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ABSTRACTS OF DOCTORAL THESES[1]

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[1] Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

SOME INVESTIGATIONS OF THE INFLUENCE OF MOISTURE CONTENT AND TEMPERATURE ON THE BIOLOGICAL DECOMPOSITION OF MATURE PLANT MATERIALS[1]

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From the Department of Agronomy, Iowa State College

Water sorption of mature plant materials from moist atmospheres and the influence of resulting moisture contents on the initiation and rate of microbial decomposition were determined. From a moisture saturated atmosphere, water sorption was found to range from 30 to 65 per cent, according to the kind of plant material. From unsaturated atmospheres, the water sorption was proportionately less and was determined by the relative humidity and temperature as well as by the previous treatment of the sorbent. Plant materials were found to absorb moisture more rapidly at high than at low temperatures. Moisture content at equilibrium, however, was greater at low than at high temperatures. It was observed that the general features of the relative humidity-moisture content relationship of the plant materials could be expressed by the mathematical equation $y = \frac{ax}{1-x} + c$ where $y =$ the moisture per cent, $x =$ the relative humidity and a and c are constants characteristic of a particular plant material.

In both the saturated and the unsaturated range the moisture content of the plant material had a pronounced influence upon the rate of microbiological decomposition. At high moisture contents decomposition proceeded rapidly, but at very low moisture contents active biological decomposition was found to be inhibited. The threshold moisture conditions for active microbiological growth varied among the several plant materials under study and are indicated as follows: for alfalfa, 20 per cent moisture and 75–80 per cent relative humidity; for sudan grass, 14–16 per cent moisture and 77–82 per cent relative humidity; for oat straw and hemp bark, 15–17 per cent moisture and 77–82 per cent relative humidity; for pine needles, 15–17 per cent moisture and 80–86 per cent relative humidity; and for sudan grass roots, 10–12 per cent moisture and 80–86 per cent relative humidity. The moisture tensions associated with the inhibition of microbiological activity are much higher than those which cause permanent wilting of field crops. The former occurs in the relative humidity range of 75–86 per cent, whereas, the latter occurs at a relative humidity of about 98.8 per cent.

[1] Doctoral thesis No. 839, submitted March 20, 1947.

The moisture in the several parts of the biological range was not equally available to the microorganisms or uniformly effective in promoting their growth. In the unsaturated range moisture increases above the threshold values resulted in a rapid and proportionate increase in microbiological decomposition. In the saturated range or at moisture contents greater than those obtaining in a saturated atmosphere successive increments of moisture had a diminishing influence on decomposition.

SOME NITROGEN AND SULFUR CONTAINING COMPOUNDS AS CHEMOTHERAPEUTIC AGENTS[1]

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General reviews were made of the chemotherapy of malaria, tuberculosis, and hyperthyroidism, and of the general methods recorded in the chemical literature for the preparation of diaryl sulfides and sulfones, quinoxalines, and 2-thiouracils. A series of each of these four types of compounds was synthesized for examination as possible chemotherapeutic agents.

Most of the recently developed drugs which have shown promise as antituberculous agents have been compounds containing the *p*-aminophenylsulfonyl group attached to an aromatic or heterocyclic ring.

The new thiophenol, *p*-isopropylthiophenol, b.p. 100-4.5°/14 mm., was prepared by the chlorosulfonation of cumene, followed by reduction of the sulfonyl chloride with zinc and dilute sulfuric acid. The chlorothiophenols were obtained from the corresponding chloroanilines. The following diphenylsulfides and sulfones, listed with their respective melting points (uncorr.), were prepared by condensing sodium thiophenoxides with the appropriate nitroaryl halides to form the nitro sulfides, which were oxidized to sulfones with hydrogen peroxide and which were reduced to the amines by an improved general method, which has heretofore been reported as impracticable due to catalyst poisoning, involving reduction with hydrogen over Raney nickel. The amines were converted to the pyrryl derivatives with acetonylacetone.

Sulfides: 2-nitro, 80.5°; 2′-methyl-2-nitro, 87–8°; 3′-methyl-2-nitro, 86–6.5°; 4′-methyl-2-nitro, 89–90°; 4-nitro, 55°; 2′-methyl-4-nitro, 64–5°; 3′-methyl-4-nitro, 47°; 4′-methyl-4-nitro, 80–1°; 2′-chloro-4-nitro, 113–4°; 3′-chloro-4-nitro, 71–1.5°; 4′-chloro-4-nitro, 83–4°; 4′-isopropyl-4-nitro, 47.5–8.5°; 3′-methyl-2,4-dinitro, 99.5–100.5°; 3′-chloro-2,4-dinitro, 108–9°; 4′-chloro-2,4-dinitro, 121–2°; 4′-isopropyl-2,4-dinitro, 95.5–6.5°; 2-amino, 43°; 2′-methyl-2-amino, 89–90.5°; 3′-methyl-2-amino, b.p./1 mm. 174–7°; 4′-methyl-2-amino, 48.5–9°; 4-amino, 95°; 2′-methyl-4-amino, 51.5–2°; 3′-methyl-4-amino, 72.5–3°; 4′-methyl-4-amino, 72–3°; 2′-chloro-4-amino, 77–8°; 3′-chloro-4-amino, 72–2.5°; 4′-chloro-4-amino, 60–1°; 4′-isopropyl-4-acetylamino, 93.5–4.5° and 108–9° (double m.p.); 3′-methyl-2,4-diamino, 112–2.5°; 3′-chloro-2,4-diamino, 94–5°; 4′-chloro-2,4-diamino, 141–2°; 4′-isopropyl-2,4-diamino, 93.5–4°; 3′-methyl-4-acetylamino, 121–2°; 3′-methyl-4-formylamino, 72.5–3.5°; 3′-methyl-4-ureido, 150–1°; 2-(2,5-dimethyl-1-pyrryl), 116–7°; 2′-methyl-2-(2,5-dimethyl-1-pyrryl), 107–8°;

[1] Doctoral thesis No. 812, submitted August 23, 1946.

4′-methyl-2-(2,5-dimethyl-1-pyrryl), 78–85°; 4-(2,5-dimethyl-1-pyrryl), 86.5–7°; 2′-methyl-4-(2,5-dimethyl-1-pyrryl), 111.5–°2; and 3′-methyl-4-(2,5-dimethyl-1-pyrryl), 66°.

Sulfones: 4-nitro, 143°; 2′-methyl-2-nitro, 144–5°; 3′-methyl-4-nitro, 121–2°; 4′-methyl-4-nitro, 171–2°; 2′-chloro-4-nitro, 160–1°; 3′-chloro-4-nitro, 142–4°; 4′-chloro-4-nitro, 182–3°; 4′-isopropyl-4-nitro, 109–11°; 3′-methyl-2,4-dinitro, 128–9°; 3′-chloro-2,4-dinitro, 155–6°; 4′-chloro-2,4-dinitro, 168–9°; 4′-isopropyl-2,4-dinitro, 118–9°; 4-amino, 174–5°; 3′-methyl-4-amino, 184.5–5.5°; 4′-methyl-4-amino, 181–2°; 2′-chloro-4-amino, 142–4°; 3′-chloro-4-amino, 189–90°; 4′-chloro-4-amino, 184–5°; 4′-isopropyl-4-amino, 154.5–5.5°; 3′-methyl-2,4-diamino, 153–4°; 4′-chloro-2,4-diamino, 200.5–1.5°; 4-(2,5-dimethyl-1-pyrryl), 153–4°; 3′-methyl-4-(2,5-dimethyl-1-pyrryl), 121–2°; and 4′-methyl-4-(2,5-dimethyl-1-pyrryl), 148–9°.

The results of all the pharmacological tests are not yet available; however, many of these compounds showed significant tuberculocidal activity.

Basically substituted quinoxalines were investigated for possible chemotherapeutic activity because of their structural relationships to known compounds possessing high activities against either malaria or tuberculosis.

Improved techniques for preparing several of the required intermediates are given. The following quinoxalines were synthesized by condensing the appropriately substituted *o*-phenylenediamines and α-dioxo compounds together: 2,3-dimethyl-6-amino, 186–7°; 6-amino-2,3-bis(*p*-methoxyphenyl), 194–6°; 6-amino-2,3-diphenyl, 172–3°; 6-amino-2,3-bis(*o*-chlorophenyl), 178–9°; 2,3-bis(*p*-nitrophenyl), 203–4°; 2,3-bis(*p*-hydroxyphenyl), 326–8°; and 6-amino-2,3-bis (*p*-hydroxyphenyl), 338–40°. 2,3-Bis(*p*-nitrophenyl)quinoxaline was reduced catalytically to 2,3-bis(*p*-aminophenyl) quinoxaline, 260–2°.

From the corresponding amines the following pyrrole derivatives were obtained: 6-(2,5-dimethyl-1-pyrryl)-2,3-dimethyl, 161–3°; 6-(2,5-dimethyl-1-pyrryl)–2,3-bis(*p*-methoxyphenyl), 189–90°; 6-(2,5-dimethyl-1-pyrryl)-2,3-diphenyl, 151–3°; and 6-(2,5-dimethyl-1-pyrryl)-2,3-bis(*o*-chlorophenyl), 211–12°. The tuberculocidal tests indicate slight to moderate activity. Information on their antimalarial activity is restricted.

The most effective antithyroid agents discovered in recent years have been derivatives of 2-thiouracil. Several new derivatives have been synthesized and are being tested for antituberculous as well as antithyroid activity.

By the alkylation of sodio acetoacetic ester, 1-diethylamino-4-carbethoxyhexanone-5, b.p. 100–7° (very largely 104–5°)/0.4 mm., and 1-(γ-diethylaminopropylmercapto)-3-carbethoxypentanone-4, b.p. 147–50°/0.4 mm., were prepared.

No products could be isolated from the condensations of the above β-oxoesters with thiourea in the presence of sodium ethoxide.

By a modification of the Claisen condensation the ethyl α-, β-, and

γ-pyridoylacetates were obtained in high yields. Modifications in the published methods of preparation of the precursory ethyl pyridoates also gave greatly improved yields. Carbethoxylation of the appropriate ketones with sodamide and ethyl carbonate gave ethyl *p*-anisoylacetate, b.p. 155–8°/0.6 mm. and ethyl β-(2-thienyl)-β-oxopropionate, b.p. 121–3°/0.4 mm.

Condensation of these β-oxoesters with thiourea in the presence of sodium ethoxide yielded 6-(α-pyridyl)-2-thiouracil, m.p. 291–4° (dec.), 6-(β-pyridyl)-2-thiouracil, m.p. 296–8° (dec.), 6-(γ-pyridyl)-2-thiouracil, dec. 355–8°, 6-(*p*-methoxyphenyl)-2-thiouracil, softens 285, melts 288–99° dec.; and 6-(β-thienyl)-2-thiouracil, dec. 293–6°.

From the corresponding substituted alkyl halides refluxed in ethanol with the monosodium salt of 6-methyl-2-thiouracil, 2-(γ-diethylaminopropylmercapto)-4-hydroxy-6-methylpyrimidine, b.p. 183–8°/0.4 mm.; 2-(*p*-nitrobenzylmercapto)-4-hydroxy-6-methylpyrimidine, m.p. 220–1°, and 2-(*p*-nitrophenethylmercapto)-4-hydroxy-6-methylpyrimidine, m.p. 224–6° (slow dec.) were secured. The attempted preparation of the amino analogs of the two nitro derivatives by catalytic reduction gave only resinous polymers.

The results of the pharmacological tests on these compounds are not yet available.

The following miscellaneous heterocyclic derivatives were synthesized: 2-*n*-butyl-4-methylpyridine, b.p. 201–2°/740 mm., its picrate, m.p. 88.5–90.5°; 2-(α-thienyl)-6-methoxyquinoline, m.p. 137–8.5°; its picrate, m.p. 190.5–2°; 1-dimethylaminomethyl-2-hydroxydibenzofuran, m.p. 114–5°; and 2-(*p*-hydroxyphenyl)-6-methoxycinchoninic acid, dec. ca. 305–10°. None of these compounds was active against either malaria or tuberculosis.

UTILIZATION OF NITROGEN BY THE ANIMAL ORGANISM
1. WHEN METHIONINE SERVES AS THE MAIN SOURCE OF NITROGEN IN THE DIET OF THE RAT[1]

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Observations in the Nutrition Laboratory of the Foods and Nutrition Department indicate that the quantity of nitrogen excreted in the urine by rats maintained on a nitrogen-low diet decreases substantially following the incorporation of dried whole eggs in the ration. This depression, contraindicated by all concepts of a constant "endogenous" nitrogen metabolism, is accompanied by the sparing of body nitrogen as calculated from nitrogen balance data, and by a restoration of the body weight lost in the previous period when the ration deficient only in nitrogenous constituents was fed. The present study reports the identification of the specific components of the dried eggs which were effective in sparing body nitrogen, an evaluation of the relative capacity of each of these compounds in regard to their ability to spare body tissue, and results of further investigation into the mechanism of body-sparing action.

The nitrogen balance test was employed, albino rats being used as the test animals. Metabolism studies were made during the period when the basal nitrogen-low diet, alone, was fed, and in a subsequent period when the basal ration was supplemented with some source of nitrogen.

The effect of feeding the basal diet and of supplementing it with certain nitrogenous constituents was evaluated in terms of the quantity of urinary nitrogen excreted, the nitrogen balance in each metabolism period, and the "body nitrogen spared," *i.e.*, the difference between the balances in the two metabolism periods. Several studies were carried out, as described below.

The feeding of a supplementary mixture of the ten essential amino acids caused a depression in the urinary excretion of nitrogen and a body-sparing effect essentially the same as that resulting from the similar administration to partially depleted rats of dehydrated eggs equivalent to 3.5 per cent of protein. The body nitrogen spared, when converted to its equivalent in body tissue, was equal to the losses in body weight prevented by supplementary feeding.

Methionine given as the sole dietary source of nitrogen to rats, following a period of feeding of the low-nitrogen diet, reduced the quantity of nitrogen excreted in the urine, and prevented a loss of body nitrogen equivalent to 5 gm. of tissue. Although the animals fed methionine failed to gain weight, they lost 5 gm. less than they would have, had no methionine been offered.

[1] Doctoral thesis No. 806, submitted July 20, 1946.

In contrast to the effect of feeding the complete amino acid mixture, a mixture composed of all the essential amino acids except methionine fed to protein-depleted rats caused a marked increase in the excretion of urinary nitrogen.

When each of the ten essential amino acids was fed separately in the routine nitrogen balance test, methionine, arginine, and histidine, only, caused a depression in the urinary excretion of nitrogen reflected in a definite sparing of body tissue. In addition to these three amino acids, leucine and lysine also spared some body tissue, seemingly exerting their effect by diminishing the excretion of nitrogen via the gut. Phenylalanine appeared to stimulate the breakdown of body tissue, as indicated by an increase in the negative nitrogen balance in the period of nitrogen feeding. Some disturbance in metabolism was evident following the feeding of tryptophane, when a yellow pigment was excreted in the urine.

Egg proteins fed to groups of rats in amounts representing 1 to 4 per cent of the quantity of the nitrogen-low ration consumed during the first balance period caused nearly identical depressions in the urinary excretion of nitrogen at each level of intake. The amount of body nitrogen spared increased with each increase in the quantity of egg proteins fed. On the other hand, the magnitude of the depression in the excretion of urinary nitrogen decreased with increasing amounts of methionine in the diet, and, interestingly, the same amount of body nitrogen was spared at each level of methionine feeding.

Dietary cystine and choline ranked very close to methionine not only in regard to the relative magnitude of the depression in the quantity of nitrogen excreted in the urine, but in the quantity of body nitrogen spared.

The quantities of nitrogen and methionine in the carcasses of animals killed at specific intervals of the balance test were determined. As a result of feeding the nitrogen-low diet for 18 days, the nitrogen content of the whole carcasses of rats decreased from 8.38 gm. to 6.41 gm. The body weight declined correspondingly. Neither the administration of methionine as a supplement to the nitrogen-low diet, nor the feeding of the nitrogen-low diet alone for an additional 11 days, had any further effect on the nitrogen content of the whole carcass. However, the addition of egg proteins to the diet for 11 days brought the nitrogen content of the carcass to 7.75 gm., the increment being equivalent to the body weight regained during this period. The per cent of nitrogen in all carcasses was the same, 2.6 per cent.

The total methionine content of the carcass fell during the first 18 days of nitrogen-low feeding, but remained constant thereafter, whether the nitrogen-low diet was fed alone or with methionine. Egg proteins given during the last 11 days of the balance period caused an increase in the total methionine corresponding to the increase in total nitrogen. The per cent of methionine nitrogen to total nitrogen in the carcasses of all groups analyzed remained constant.

The livers of animals fed a nitrogen-low diet for 29 days contained

slightly less nitrogen than did those of animals whose diets were supplemented in the last 11 days of the nitrogen balance test with methionine. The ratio of methionine nitrogen to total nitrogen was the same in the livers of both groups, 3.0 per cent.

The concentration of methionine per gram of dry, fat-free muscle was constant under all conditions of dietary manipulation.

The fat content of muscle tissue was constant despite dietary changes. Hepatic tissue reflected the dietary lack of nitrogen by a rise in the concentration of fat, a condition not corrected by the feeding for 11 days of supplementary egg proteins, choline, or a mixture of the ten essential amino acids. Supplementary methionine and cystine in the ration caused a marked increase in the fat content of the liver.

Thus, it seems that the effect of the introduction of egg proteins into a ration deficient in protein on the nitrogen metabolism of the rat may be ascribed to the presence of the essential amino acids. Methionine, which is the most powerful in sparing body protein, probably acts largely through its capacity to form cystine, choline, or related compounds. No single amino acid, however, can be very important quantitatively in sparing body tissue in the partially depleted animal, in view of the complex demands of the body for the synthesis of its structural and functional components. Egg proteins and a mixture of the essential amino acids are much more effective in this respect than is any individual amino acid, as indicated by the quantities of body nitrogen spared and by restoration of weight lost when the nitrogen-poor ration was fed.

Analyses of whole carcass, liver, and muscle of adult rats suggest that the effect of methionine on nitrogen metabolism does not represent the over-all picture of nitrogen metabolism, wherein amino acids in the metabolic pool arising from food and tissue sources are used in the maintenance of body tissue and in the synthesis of functional proteins and of nitrogenous and non-nitrogenous metabolites. Continued loss in body weight, the lack of change in the total nitrogenous content of the carcass, and the relatively small increment in labile reserves in the liver when methionine is fed to the depleted animal, suggest that methionine does not act in the maintenance of body tissues. The powerful effect of dietary methionine and cystine and the somewhat less pronounced influence of arginine and histidine in decreasing the losses of nitrogen in the urine of animals maintained on a nitrogen-low diet are evidence that the body does raid its tissues for specific metabolites for the synthesis of specific functional substances.

SOME DERIVATIVES OF γ-VALEROLACTONE[1]

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Some of the reactions of γ-valerolactone and its reduction product, 1,4-pentanediol, have been examined in anticipation that new derivatives might prove worthy of industrial applications. Attention was directed to the possiblity of using γ-valerolactone and 1,4-pentanediol as starting points in the synthesis of bifunctional acids and amines from which linear polyamides of the "nylon" type could be prepared. One phase of the study proved to be a new approach to the synthesis of β-alkoxypropionic acid and necessitated an extension of the investigation to a series of structurally related compounds.

In verification of the work of Eijkman (1), γ-valerolactone was found to react with benzene in the presence of aluminum chloride to yield 61 per cent of γ-phenylvaleric acid (*p*-bromophenacyl ester, m.p. 76°). Proof that γ-phenylvaleric acid prepared in this way was identical with that obtained by other methods (2,3,4,5) was afforded by ring closure to 4-methyl-1-tetralone (3,5) and preparation of 4-methyl-1-tetralone semicarbazone. (2,3,5)

Attempts to condense benzene with two molecular equivalents of γ-valerolactone gave mixtures from which, subsequent to esterification, there was isolated diethyl γ,γ′-phenylene-divalerate of boiling point 165–169°/0.5 mm., d_4^{25} 1.0212, and n_D^{25} 1.4914. This compound was not obtained as the free acid, nor was the orientation of the substituents in the benzene ring determined.

Improved yields were obtained in the re-examination of some previously reported (6,7,8) methods for the preparation of 1,4-pentanediol by catalytic hydrogenation. The reactions were carried out without solvent in the presence of copper-chromium oxide catalyst. γ-Valerolactone, at 300 atmospheres and 250–290°, gave 32–83 per cent of 1,4-pentanediol. Lower yields of the glycol were obtained at the higher reduction temperatures and from these preparations 2-methyltetrahydrofuran was isolated and identified. Ethyl levulinate, at 250° and 250 atmospheres' pressure, gave 72 per cent of 1,4-pentanediol. Levulinic acid, at 267 atmospheres and 245–300°, yielded 11 per cent of γ-valerolactone and 44 per cent of 1,4-pentanediol.

1,4-Pentanediol was treated with acrylonitrile in the presence of 40 per cent aqueous potassium hydroxide (9) to yield 83 per cent of 1,4-di-(2-cyanoethoxy)-pentane of boiling point 157°/1 mm., n_D^{28} 1.4478, and d_4^{28}

[1] Doctoral thesis No. 809, submitted July 22, 1946.

1.005. This compound was characterized by conversion to the solid *bis*-(ethyliminoester hydrochloride) melting with decomposition at 103–104°.

A series of 14 β-alkoxypropionitriles was prepared by the addition of acrylonitrile to appropriate alcohols (10) and glycols (9). Six of these compounds were prepared for the first time. The yields and physical constants were as follows: β-*n*-propoxypropionitrile, 83 per cent, b.p. 87–89°/24 mm. or 84°/19 mm., d_4^{20} 0.8988, n_D^{20} 1.4131; β-*iso*-butoxypropionitrile, 81 per cent, b.p. 88–90°/19 mm. or 91°/20 mm., d_4^{20} 0.8821, n_D^{20} 1.4143; β-*sec*-butoxypropionitrile, 79 per cent, b.p. 90°/19 mm., d_4^{20} 0.8880, n_D^{20} 1.4156; β-*iso*-amyloxypropionitrile, 82 per cent, b.p. 99°/13 mm., d_4^{20} 0.8818, n_D^{20} 1.4218; β-*sec*-amyloxypropionitrile, 70 per cent, b.p. 98°/16 mm., d_4^{20} 0.8844, n_D^{20} 1.4205; β-allyloxypropionitrile, b.p. 95°/24 mm. or 92°/19 mm., d_4^{20} 0.9380, n_D^{20} 1.4330.

1,4-Di-(2-cyanoethoxy)-pentane was hydrogenated at 100–120° and 130–200 atmospheres in the presence of Raney nickel and liquid ammonia to give 77–82 per cent of 1,4-di-(3-aminopropoxy)-pentane of boiling point 132–135°/2 mm., d_4^{24} 0.9448, and n_D^{24} 1.4596. The diamine was miscible with water in all proportions and its 5 per cent aqueous solution exhibited a pH in excess of 11. The picrate and hydrochloride of 1,4-di-(3-aminopropoxy)-pentane were obtained as uncrystallizable oils.

From 1,4-di-(3-aminopropoxy)-pentane a series of normal and acid salts was prepared using monobasic and dibasic acids. The diacetate, dipropionate, dibutyrate, dilevulinate, dibenzoate, malonate, glutarate, itaconate, dimalonate, diglutarate, and diphthalate were obtained as uncrystallizable oils or gums. The solid salts and their melting points were as follows: disulfamate, about 65°; oxalate, 196–198°; succinate, 121–122°; adipate, 136–138°; sebacate, about 50°; maleate, 129–131°; phthalate, 125–126°; dioxalate, 108–110°; disuccinate, 92–94°; diadipate, 102–103°; and dimaleate, 80–85°. These salts were very soluble in water and, with the exception of the normal oxalate, easily soluble in absolute ethanol.

The saponification of 1,4-di-(2-cyanoethoxy)-petane, di-(2-cyanoethyl)-ether, and β-ethoxypropionitrile by means of 9 per cent to 22 per cent sodium hydroxide gave complex mixtures. From the basic hydrolysis of β-ethoxypropionitrile there was isolated a substance which displayed some of the properties of acrylic acid. A mechanism is suggested for the decomposition of β-alkoxypropionitriles under conditions of basic hydrolysis.

Hydrolysis of β-alkoxypropionitriles with concentrated hydrochloric acid at 70–100° was found to be a fairly general method for the preparation of β-alkoxypropionic acids. Ten monobasic β-alkoxypropionic acids were prepared and characterized as *p*-bromophenacyl esters. Yields,

physical constants for hitherto unreported compounds, and data concerning the derivatives were as follows: β-Ethoxypropionic acid, (11) 86 per cent, (*p*-bromophenacyl ester, large leaflets, m.p. 47–48°); β-*n*-propoxypropionic acid, (12) 80 per cent, (*p*-bromophenacyl ester, shiny leaflets, m.p. 57–58°); β-*iso*-propoxypropionic acid, (12) 60 per cent, (*p*-bromophenacyl ester, platelets, m.p. 44–44.5°); β-*n*-butoxypropionic acid, 69 per cent, b.p. 96–97.5°/1 mm., n_D^{20} 1.4268, d_4^{20} 0.9929, (*p*-bromophenacyl ester, shiny leaflets, m.p. 55°); β-*iso*-butoxypropionic acid, 67 per cent, b.p. 88–89°/1 mm., n_D^{20} 1.4227, d_4^{20} 0.9843, (*p*-bromophenacyl ester, fibrous needles, m.p. 57.5–58.5°); β-*sec*-butoxypropionic acid, 56 per cent b.p. 90–91.4°/1 mm., n_D^{20} 1.4252, d_4^{20} 0.9946, (*p*-bromophenacyl ester, oil); β-*iso*-amploxypropionic acid, (13,14) 69 per cent, (*p*-bromophenacyl ester, glistening needles, m.p. 56°); β-*sec*-amyloxypropionic acid, 49 per cent, b.p. 100–101.4°/1 mm., n_D^{20} 1.4289, d_4^{20} 0.9833, (*p*-bromophenacyl ester, oil); β-allyloxypropionic acid, 33 per cent, b.p. 84°/1 mm. or 111–112°/ 6 mm., n_D^{20} 1.4423, d_4^{20} 1.0604, (*p*-bromophenacyl ester, platelets, m.p. 38–39°); β-(2-methoxyethoxy)-propionic acid, 75 per cent, b.p. 110°/0.5 mm., n_D^{20} 1.4356, d_4^{20} 1.1146, (*p*-bromophenacyl ester, oil, m.p. about 15°).

The same hydrolysis procedure served for the preparation of four dibasic acids of the β-alkoxypropionic type. These were characterized by conversion to simple amides *via* the acid chlorides which were not isolated. The acids and their derivatives were as follows: di-(2-carboxyethyl)-ether, 97 per cent, m.p. 60–61°, b.p. 189–192°/less than 1 mm., (amide, (9) m.p. 144°); 1,2-di-(2-carboxyethoxy)-ethane, 94 per cent, m.p. 66°, (amide, (15) m.p. 123°); 1,3-di-(2-carboxyethoxy)-propane, 90 per cent, m.p. 86–87°, (amide, m.p. 124°); di-(2-[2-carboxyethoxy]-ethyl)-ether, 91 per cent, undistillable, uncrystallizable oil, (amide, (15) m.p. 103–103.5°).

1,4-Di-(2-cyanoethoxy)-pentane underwent a partial cleavage during treatment with concentrated hydrochloric acid at 75–100°. From the resulting mixture there was isolated, subsequent to esterification, a chlorine-containing compound of boiling point 109–112°/ 2.5 mm., d_4^{25} 1.0325, and n_D^{25} 1.4386. The complete analysis of this compound was in close agreement with that calculated for an ethyl β-(chloroamyloxy)-propionate.

1,4-Di-(2-carbethoxyethoxy)-petane (b.p. 151–153°/1 mm. d_4^{25} 1.0174, n_D^{25} 1.4363) was prepared by two methods, both of which involved the hydrolysis, at room temperature, of an initially-formed mineral acid salt of the *bis*-ethyliminoester of 1,4-di-(2-cyanoethoxy)-pentane.

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THE INTRODUCTION OF FUNCTIONAL GROUPS INTO SOME ORGANOSILANES[1]

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It was the primary purpose of this thesis to prepare organosilanes which contained functional groups and which so lent themselves to more fundamental studies. In particular, one of the broader purposes was to investigate the general relationships between the chemistry of similar silicon and carbon molecules.

Tetraphenylsilane was synthesized by the addition of four equivalents of phenyllithium to one of either silicon tetrachloride or tetraethoxysilane or tetrathioethoxysilane. The yields varied from 97–99 per cent; the time of reaction was only that required to complete addition of the reagents. Similarly, the following compounds were prepared: tetraethylsilane (92 per cent), b.p., 150–151°/739 mm.; tetra-*n*-butylsilane (97 per cent), b.p., 156–157°/739 mm.; d_4^{20} 0.8008; n_D^{20} 1.4465; MR_D calcd. 85.49; MR_D found 85.19.

Tetraisopropylsilane could not be prepared by this general method; treatment of silicon tetrachloride or tetraethoxysilane with four equivalents of isopropyllithium gave triisopropylchlorosilane and triisopropylethoxysilane, respectively. Their constants were: triisopropylchlorosilane, b.p., 198°/739 mm.; d_4^{20} 0.9008; n_D^{20} 1.4518; MR_D calcd. 58.00; MR_D found 57.81; triisopropylethoxysilane, b.p., 200°/738 mm.; d_4^{20} 0.8657; n_D^{20} 1.4560; MR_D calcd. 63.37; MR_D found 63.18. Triisopropylchlorosilane reacted with ethanol in the presence of pyridine to give triisopropylethoxysilane (95 per cent).

Triphenyl-*p*-tolylsilane (91 per cent), m.p. 134–135°, was synthesized by the careful addition of three equivalents of phenyllithium to one of silicon tetrachloride followed by the addition of one equivalent of *p*-tolyllithium. Similarly, these compounds were prepared: tri-*p*-tolylphenylsilane (89 per cent), m.p. 127–128°; triphenylmethylsilane (92 per cent), m.p. 66–67°; trimethylphenylsilane (89 per cent), b.p., 163–164°/737 mm.; n_D^{20} 1.4890.

When trimethylphenylsilane was synthesized by the addition of one equivalent of methyllithium to one of silicon tetrachloride followed by the addition of three equivalents of phenyllithium the yield was only 23 per cent.

[1] Doctoral thesis No. 826, submitted December 16, 1946.

Diphenyldi-*p*-tolylsilane (78 per cent), m.p. 176–177°, was prepared by adding two equivalents of phenyllithium to one of silicon tetrachloride and then adding two equivalents of *p*-tolyllithium.

The action of three equivalents of phenyllithium upon one of silicon tetrachloride gave a reaction mixture which was hydrolyzed to give triphenylsilanol (97 per cent), m.p. 150–151°.

Triphenylethoxysilane (68 per cent), m.p. 64–65°, was synthesized by reacting three equivalents of phenyllithium with one of tetraethoxysilane. Hexaphenyldisiloxane (28 per cent), m.p. 220–221°, was also isolated from this reaction.

p-Bromophenyltrimethylsilane (73 per cent) was prepared by first adding one equivalent of *p*-bromophenylmagnesium bromide to one of triethoxychlorosilane and then adding three equivalents of methyllithium to this reaction mixture, b.p., 94–95°/2.5 mm.; d_4^{20} 1.2023; n_D^{20} 1.5272; MR_D calcd. 57.52; MR_D found 57.48. This compound reacted with lithium to give the corresponding organolithium compound. Carbonation of this derivative gave *p*-carboxyphenyltrimethylsilane (60 per cent), m.p. 110–111°.

Benzyltriethoxysilane (65 per cent), b.p. 245–248°/739 mm., was synthesized by the action of one equivalent of benzylmagnesium chloride upon one of triethoxychlorosilane. This same procedure was used to prepare phenyltriethoxysilane (66 per cent), b.p. 228–230°/741 mm.

Benzyltriethoxysilane, when treated with three equivalents of methyllithium, gave benzyltrimethylsilane (93 per cent), b.p., 185–187°/736 mm.

When benzyltriethoxysilane was treated with three equivalents of methylmagnesium iodide there was obtained *sym.*-dibenzyltetraethyldisiloxane (79 per cent), b.p., 318–320°/735 mm.; d_4^{20} 0.9991; n_D^{20} 1.5209; MR_D calcd. 97:20; MR_D found 97.01.

Bromination of trimethylbenzylsilane gave benzyl bromide; whereas nitration gave phenylnitromethane.

Triethylchlorosilane and ethyl sodioacetoacetate gave ethyl β-triethylsiloxycrotonate (61 per cent), b.p., 108–110°/6 mm.; d_4^{20} 0.9590; n_D^{20} 1.4560; MR_D calcd. 69.12; MR_D found 69.42.

These variously substituted β-lactams of β-anilino-β-phenylpropionic acid were prepared by using the general procedure of Gilman and Speeter[2]: β-lactam of β-anilino-β-*p*-anisylpropionic acid (25 per cent), m.p. 154–155.5°; β-lactam of β-*p*-chloroanilino-β-*o*-chlorophenylpropionic acid (38 per cent), m.p. 129–130°; β-lactam of β-*p*-chloroanilino-β-*p*-anisylpropionic acid (36 per cent), m.p. 108–109°; β-lactam of β-*m*-chloroanilino-β-*p*-anisylpropionic acid (17.5 per cent), m.p. 110.5–112°; β-lactam of β-*p*-chloroanilino-β-phenylpropionic acid (49.6 per cent), m.p. 110–111°; β-lactam of β-*p*-anisidino-β-phenylpropionic acid (43 per cent), m.p. 96°;

[2] Gilman and Speeter, Jour. Am. Chem. Soc., 65, 2256 (1943).

and the β-lactam of β-anilino-β-*o*-chlorophenylpropionic acid (34 per cent), m.p. 93–94°.

The β-lactam of β-anilino-β-phenylpropionic acid was hydrolyzed by means of aqueous base (25 per cent) or acid (45 per cent); the product in both cases was β-anilino-β-phenylpropionic acid, m.p. 134–135°.

An ethanol solution of the β-lactam of β-anilino-β-phenylpropionic acid when treated with dry hydrogen chloride gave ethyl β-anilino-β-phenylpropionate (85 per cent), m.p. 74–75°.

Nitration of 2-phenylpyridine[3] by the method of Forsyth and Pyman[4] gave a mixture of the 2-(nitrophenyl) pyridines which were separated according to the method of these investigators. 2-(4′-Nitrophenyl) pyridine was reduced by hydrogen and Raney nickel to 2-(4′-aminophenyl) pyridine (95 per cent), m.p. 97–98°.

2-(4′-Aminophenyl) pyridine with benzaldehyde gave 2′-(4′-benzalaminophenyl) pyridine (95 per cent), m.p. 102–103°; with salicylaldehyde, 2-(4′-salicylalaminophenyl) pyridine (93 per cent), m.p. 137–138°; with acetonylacetone, 2-[4′-(2″,5″-dimethylpyrryl-1″) phenyl]pyridine, m.p. 98.5–99°; with formic acid, 2-(4′-formylaminophenyl) pyridine, m.p. 127–128°; and with acetic anhydride, 2-(4′-acetaminophenyl) pyridine (94 per cent), m.p. 134–135°.

Nitration of 2-(4′-acetaminophenyl) pyridine gave 2-(3′-nitro-4′-acetaminophenyl) pyridine (94 per cent), m.p. 142–143°. Basic hydrolysis of this product gave 2-(3′-nitro-4′-aminophenyl) pyridine (91 per cent), m.p. 148–149°. Action of formic acid in this latter product gave 2-(3′-nitro-4′-formylaminophenyl) pyridine (82 per cent), m.p. 148–149°.

2-(4′-Methoxyphenyl) pyridine[3] was hydrolyzed with hydrobromic acid to give 2-(4′-hydroxyphenyl) pyridine (90 per cent), m.p. 164–165°. Nitration of this product gave 2-(3′-nitro-4′-hydroxyphenyl) pyridine (88 per cent), m.p. 126–127°.

p-Bromothiophenol (37.5 per cent), m.p. 74–75°, was prepared by the action of potassium ethylxanthate upon a *p*-bromobenzenediazonium chloride solution. This product upon treatment with *n*-butyllithium gave *p*-mercaptophenyllithium; carbonation of this compound gave *p*-mercaptobenzoic acid (70 per cent), m.p. 213–215°. *p*-Mercaptophenyllithium reacted with pyridine to give 2-(4′-mercaptophenyl) pyridine (21 per cent), b.p. 190–193°/3 mm.

γ-(*p*-Nitrophenyl) propyl chloride[5] reacted with diethylamine to give diethyl-[γ-(*p*-nitrophenyl) propyl]amine (70 per cent), b.p., 145–146°/1 mm. This product was reduced with hydrogen and Raney nickel to give diethyl-[γ-(*p*-aminophenyl) propyl]amine (92 per cent), b.p., 152–153°/2 mm.

The isomeric 2-nitrophenylpyridines, 3-nitrophenylpyridines, and 4-nitrophenylpyridines were synthesized by a modification of the method of Heilbron and co-workers[6].

[3] J. T. Edward, unpublished studies.
[4] Forsyth and Pyman, Jour. Chem. Soc., 2912 (1926).
[5] Braun and Deutsch, Ber., 45, 2504 (1912).
[6] Haworth, Heilbron and Hey, Jour. Chem. Soc., 349 (1940).

2-(2′-Nitrophenyl) pyridine was reduced to the corresponding amine (89 per cent), b.p. 192–194°/1 mm. These derivatives of this amine were formed: 2-[2′-(2″,5″-dimethylpyrryl-1″) phenyl]pyridine, m. p. 101–102°; and 2-(2′-salicylalaminophenyl) pyridine, m.p. 118–120°.

3-(2′-Nitrophenyl) pyridine was reduced to the corresponding amine (90 per cent), b.p. 210–212°/3 mm. This compound gave 3-[2′-(2″,5″-dimethylpyrryl-1″) phenyl]pyridine, m.p. 66–67°.

4-(3′-Aminophenyl) pyridine, m.p. 164–166°, 3-(3′-aminophenyl) pyridine, m.p. 77–78°, and 2-(3′-aminophenyl) pyridine, m.p. 72–73°, were prepared from the corresponding nitro-compounds.

2-(3′-Aminophenyl) pyridine gave 2-[3′-(2″,5″-dimethylpyrryl-1″) phenyl]pyridine, m.p. 83.5–84°, and 2-(3′-salicylalaminophenyl) pyridine, m.p. 77–78°.

2,6-(4′,4″-Diaminodiphenyl) pyridine, m.p. 216–218°, and 3-(4′-aminophenyl) pyridine, m.p. 116–118°, were prepared from their corresponding nitro-compounds. This latter product gave 3-[4′-(2″,5″-dimethylpyrryl-1″) phenyl]pyridine, m.p. 116–117°.

2-(2′-Nitro-4′-methoxyphenyl) pyridine (1.3 per cent), m.p. 89–90°, and 3(?)-(2′-nitro-4′-methoxyphenyl) pyridine (2.3 per cent), m.p. 66–67°, were prepared by the action of a 2-nitro-4-methoxybenzenediazonium chloride solution on pyridine.

Reduction of the 2-isomer gave 2-(2′-amino-4′-methoxyphenyl) pyridine (80 per cent), m.p. of dihydrochloride 199–200°.

2-(4′-Nitrophenyl) thiophene, prepared by a modification of the method of Gomberg and Bachman[7], on reduction with hydrogen and Raney nickel gave 2-(4′-aminophenyl) thiophene, m.p. 69–71°. The position of the phenyl ring in the thiophene nucleus was proven by deamination of 2-(4′-aminophenyl) thiophene to the known 2-phenylthiophene.

[7] Gomberg and Bachman, Jour. Am. Chem. Soc., 46, 2339 (1924).

THE REACTION OF IODINE WITH STARCH AND THE SCHARDINGER DEXTRINS[1]

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The reaction of iodine with amylose has been explained in terms of the helical theory. This investigation offers additional evidence to support the helical theory. The reaction of the Schardinger dextrins and iodine has been studied because of the similarity between the ring structure of the dextrins and the helical structure of the amylose molecule.

The reactions of iodine, iodide ion and the Schardinger dextrins were followed potentiometrically by measuring the E. M. F. of a cell of the type

$$(Pt), I_2(c_1), I^-(m_1), A : I_2(c_2), I^-(m_2), (Pt)$$

where A is the material studied. The results indicate that the following reactions occur in solution between cyclohexaamylose (α-dextrin), iodine and iodide ion:

$$\alpha + I_2 \rightarrow \alpha I_2 \qquad (1)$$

$$\alpha I_2 + I^- \rightarrow \alpha I_3^- \qquad (2)$$

$$\alpha + I^- \rightarrow \alpha I^- \qquad (3)$$

$$\alpha I^- + I_2 \rightarrow \alpha I_3^- \qquad (4)$$

The equilibrium constants for these reactions are: $K_1 = 2.0 \times 10^{+3}$, $K_2 = 1.35 \times 10^{+5}$, $K_3 = 13.5$, $K_4 = 2.0 \times 10^{+7}$. The crystalline complex formed between cyclohexaamylose and potassium iodide has been prepared. The constitution of the crystalline iodine complexes of cyclohexaamylose, which have previously been reported, agree with the above reactions.

An identical series of reactions with cycloheptaamylose (β-dextrin). The equilibrium constants for the reactions

$$\beta + I^- \rightarrow \beta I^- \qquad (3^*)$$

$$\beta I^- + I_2 \rightarrow \beta I_3^- \qquad (4^*)$$

are $K_3 = 1.45$ and $K_4 = 1.0 \times 10^{+6}$.

The results obtained with the Schardinger dextrins have been used to interpret the reactions between amylose, iodine and iodide ion. These

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reactions were also studied potentiometrically. The characteristic behavior of the amylose-iodine complex is best explained by the series of reactions:

$$\mathrm{Am\ (aq) + nI_2\ (aq) \rightarrow Am(I_2)_n\ (aq)}$$

$$\mathrm{Am(I_2)\ (aq) + nI^-\ (aq) \rightarrow Am(I_3^-)_n} \qquad \mathrm{(s)}$$

$$\mathrm{Am\ (aq) + nI^-\ (aq) \rightarrow Am(I^-)_n\ (aq)}$$

$$\mathrm{Am(I^-)_n\ (aq) + nI_2\ (aq) \rightarrow Am(I_3^-)_n} \qquad \mathrm{(s)}.$$

The effect of a change in the iodide concentration, amylose concentration, or iodine concentration is explained by the above reactions. The following thermodynamic quantities have been determined for the reaction,

$$\mathrm{Am\ (aq) + nI_2 + nI^- \rightarrow Am(I_3^-)_n\ \ (s)},$$

per mole of iodine:

$\Delta F^\circ_{298} = -12{,}000$ cals. per mole of iodine
$\Delta S^\circ_{298} = -25.6$ e. u. per mole of iodine
$\Delta H^\circ_{298} = -19{,}600$ cals. per mole of iodine.

The effect of chain length on the iodine activity of the amylose-iodine complex has been used as the basis for the development of a method of determining the chain length distribution of amylose. The weight-average distribution of amylose can be determined from a single potentiometric iodine titration. The method should be useful in determining the efficiency of fractionation procedures.

THE QUANTITATIVE SPECTROGRAPHIC ANALYSIS OF THE RARE EARTH ELEMENTS[1]

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The limited application of ordinary chemical methods to the analysis of the rare earth group has resulted in the development of a number of physical and physico-chemical methods. None of the methods so far proposed have offered a practical means of performing the following analyses: (a) the determination of low concentrations of rare earth impurities in purified rare earths, and (b) the determination of major amounts of those members of the group which possess no absorption bands in the wavelength regions accessible by ordinary spectrophotometric apparatus. This investigation is concerned with development of emission spectra methods which are capable of yielding the analytical information mentioned above.

A Jarrell-Ash Wadsworth mounting 21-foot grating spectrograph was used. The complex spectra of the rare earths necessitated the use of the second order in which the linear dispersion was 2.5 Å/mm. The external optical system was designed to focus the source on the collimating mirror. A rotating stepped sector placed in front of the slit was used for intensity modulations. The plates were processed in an ARL-Dietert developing machine and photometric measurements were made on an ARL-Dietert comparator-densitometer. The calibration of the response of the Eastman Type III-O and Spectrum Analysis No. 1 emulsions was made by the two-step sector, preliminary curve method.

The logical choice of the chemical form of the samples for excitation was the ignited rare earth oxides since the samples from fractionation procedures are obtained either in this form or are readily converted to oxides. The refractory properties of the rare earth oxides restricted the type of excitation to the d-c arc, for it alone produced temperatures high enough to vaporize the oxides into the discharge zone. Since the erraticalness and poor reproducibility of the d-c arc discharge greatly impairs the usefulness of this source for quantitative analysis, the plan of attack was to develop means of reducing and correcting for the large excitation variables.

It was found that d-c arcs of currents as high as 18 amperes did not provide electrode temperatures high enough to vaporize a sufficient amount of the rare earth oxides to maintain a stabilized arc. Following the suggestions of Bauer, a number of experiments were performed in which the rare earth oxides were mixed with metallic oxides of lower

[1] Doctoral thesis No. 851, submitted June 9, 1947.

boiling points in the hope that the latter would support a stabilized arc and aid in transporting the rare earth oxides into the discharge zone during the stable arc period. These added oxides successfully stabilized the 17.3 ampere arc but moving-plate spectrograms taken during the excitation revealed that the major portion of the rare earths were actually volatilized and excited immediately preceding and during the unstable discharge following the stable period. A mixture of powdered graphite and rare earth oxides was found to produce a stabilized arc at 16 amperes or above. Moving-plate spectrograms of these arcings indicated uniform emission during the excitations. Apparently the more volatile rare earth metals were formed either by chemical reduction or by carbide formation followed by thermal decomposition of the latter. The optimum experimental conditions for this type of excitation were: current, 17.5 amperes; weight of electrode charge, 15 mg; type of electrode, ¼-inch diameter graphite with 2 mm-deep thin-walled cavity; composition of charge, 1:1 mixture of rare earth oxides and powdered graphite.

The internal standard technique was used to compensate for the residual excitation and photographic variables. In this technique it is assumed that concentration is a function of the single variable I_A/I_B, where I_A and I_B, respectively, are the line intensities of the element to be determined and the internal standard element. Accordingly, for greatest effectiveness, it was desirable to choose the internal standard and line pair so as to maintain a constancy of I_A/I_B during the excitation, regardless of changes in voltage, arc current, effective excitation temperature, distillation and other effects. In view of the similarity in the physical properties of the rare earths, the logical choice of the internal standard was a rare earth which was either present or could be introduced into a sample in a constant amount. For the determination of minor impurities in purified rare earths, this constant amount was provided by the major constituent whose concentration remained essentially constant. The selection of cerium as the internal standard for the analysis of complex mixtures was based on the relative ease with which tetravalent cerium could be separated from the rest of the rare earths. The presence of a constant rare earth concentration in mixtures for internal standard purposes was assured by first removing any cerium present and then reintroducing a standard amount. The following advantages were obtained by the addition of the cerium so that the final composition was 80 per cent ceric oxide and 20 per cent sample: (a) elimination of multiple cerium separations since the cerium remaining after the preliminary separation was negligible compared to the amount added, (b) elimination of the preliminary cerium separation when the original cerium concentration was low, (c) reduction of extraneous influences on the excitation conditions of large changes in sample compositions, and (d) reduction of self-reversal effects on the lines of the elements to be determined.

Insofar as possible, line pairs were selected whose components possessed equivalent excitation properties and intensities and proximity of wavelengths. The experimental determinations of constancy of intensity

ratios during the excitations were made by means of moving-plate spectrograms. The following mixtures were investigated: (a) neodymium oxide containing a small amount of samarium oxide; (b) samarium oxide containing a small amount of europium oxide; and (c) a mixture of equal amounts of yttrium and gadolinium oxide plus the added ceric oxide. The relatively small change in the intensity ratios for mixtures (b) and (c) approached the idealized condition seldom attained in d-c arc methods. For (a) the ratio change was greater but subsequent tests on reproducibility of the integrated ratio indicated an average deviation of 3 to 4 per cent and thus low enough for quantitative application.

The quantitative calibrations were performed by exposing synthetic standards under the optimum experimental conditions. The calibration curves were obtained by plotting the intensity ratios of the selected line pairs versus concentration on log-log paper. Calibrations were made for the following determinations: (a) minor amounts (0.1–4.0 per cent) of samarium in neodymium, (b) minor amounts (0.01–2.0 per cent) of europium in samarium, and (c) major amounts (10–100 per cent) of yttrium and gadolinium in complex mixtures.

The successful application of the internal standard principle was shown by the negligible effect on the intensity ratios of large variations in the experimental conditions. The results from a series of accuracy and precision determinations indicated an experimental error of 3.5 to 4 per cent.

The methods which were developed have low time and sample requirements per analysis and are readily adaptable to the analysis of purified rare earths and complex mixture.

THE EFFECT OF CROP ROTATION ON SOIL AGGREGATION AND RELATED CHARACTERISTICS IN A BELINDA SILT LOAM SOIL[1]

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Soil aggregate studies were made on samples from 8 different rotations at the pasture farm near Albia, Iowa, located on Belinda silt loam soil. The rotation experiments were started in 1941. The eight types of rotations were as follows: corn-oats, corn-oats with sweet clover as catch crop, corn-oats-meadow, corn-oats-meadow-meadow, corn-corn-oats-meadow-meadow, corn—continuously with a sweet clover cover crop, corn-soybeans, and corn-corn-oats-meadow.

The seasonal variation and the effect of rotation on total aggregates, size distribution, aggregate stability and permeability may be summarized as follows:

In the 8 sampling dates from March, 1945 to September, 1946 the highest percentage of stable aggregates > .1 mm. is from the sampling of August, 1945, the lowest being from that of March of the same year. The seasonal variation follows a regular trend for all sized fractions. In general, as the larger sized fraction decreased the smaller sized increased. For instance, from July to December in 1945 the largest fraction was high while the smallest fraction was low.

The low moisture content of the soil which followed a period of rapid decomposition of organic matter, in addition to the presence of rootlets from the rapidly growing crop are the important factors contributing to the high value of > .1 mm. aggregates in August, 1945.

The moisture content at the time of sampling varied widely between dates and under different crops. This difference amounted to 50 per cent between the oats and clover plots and corn soybean plots in September, 1946, which undoubtedly, greatly affected the difference in size distribution of soil aggregates between these plots.

The effect of different rotations on total aggregates > .1 mm. was in the order corn-oats-meadow-meadow > corn-corn-oats-meadow-meadow > corn-corn-oats-meadow > corn-oats with sweet clover catch crop > corn-oats > corn-soybean.

The effect of different rotations on size distribution of aggregates was very striking. In continuous grass plots the > 2 mm. fraction predominates over the smaller sized aggregates, especially the smallest fraction of .25–.1 mm. A reverse condition was found for the other rotations; that is, the smallest fraction, .25–.1 mm., predominated over the > 2 mm. fraction. A corn-oats-meadow-meadow rotation gave the highest percentage of > 2mm. fraction, whereas the reverse was true for the corn-soybean

[1] Doctoral thesis No. 835, submitted March 18, 1947.

rotation. There was an insignificant change in the 1–.5 and .5–.25 mm. fractions.

The crop grown for the current year as well as the intensity of the rotation affected the size distribution of aggregates, especially the > 2 mm. and > 2–1 mm. fractions.

The effect of an individual crop in the previous year on the size distribution of soil aggregates was also noticeable. For instance, the second year meadow in corn-oats-meadow-meadow rotation is much higher than that of the first year corn in corn-corn-oats-meadow rotation.

The total stable aggregates > .1 mm. were increased by air drying. In spite of the fact that air drying caused a decrease in the > 2 mm. and 2–1 mm. fractions, there was a sufficient increase in the .5–.25 mm. and the .25–.10 mm. fractions to account for a total increase in aggregates larger than .1 mm. In general, air drying helps to consolidate the smaller fractions and tends to break down the larger ones, partly at least, by weakening the binding strength of the fibrous rootlets that were present in soil, especially in the > 2 mm. fraction.

Since the aggregates > 2 mm. fraction as well as the finest aggregates .25–.1 mm. were both important factors in characterizing soil structure the ratio between these fractions was proposed as a valuable criterion in ranking the different rotations on the basis of development of desirable structure.

Different rotations as well as different crops on the land when the samples were taken affected greatly the stability of aggregates. A comparison of the April 4 and September 17, 1946 dates showed that the changes occurred in soil aggregation as the growing season advanced. For example, in the corn-oats-meadow rotation the stability factor under first year meadow on April 4, 1946 was .060; on September 17, when the meadow was in its second year the stability factor was .180.

A highly significant correlation coefficient of .99 was found between "stability factor" and permeability for samples of April 4 and also a highly significant correlation coefficient of .90 was found for the samples of September 17, 1946. The determination of "stability factor" and permeability was made on the 2–1 mm. fraction. It was thus concluded that permeability was closely related to stability of individual aggregates.

The highest carbon dioxide evolution was found under grass regardless of sampling dates. The lowest was found under corn-oats and corn-soybean rotations.

The organic carbon content under grass or corn-meadow rations was higher than that under corn-soybean rotation. It was concluded that the high carbon dioxide evolution and the high organic carbon content in samples from grass or meadow plots were closely related to the high percentage of total stable aggregates > .1 mm., which in turn was the result of a higher percentage of the > 2 mm. and 2–1 mm. fractions in the soil under grass or meadow.

The volume of roots from the surface 6 inches of soil under different grasses was in the order Kentucky blue grass > timothy > big blue stem

> orchard grass > brome grass > red top. This was not in agreement with data reported by some investigators that showed an appreciably higher volume of roots under brome grass than under Kentucky blue grass. It was also of particular interest to note that while the red top had the lowest volume of grass roots the soil under red top contained the highest percentage of stable aggregate. In contrast with this the Kentucky blue grass, which had the highest volume of grass roots was found to have the lowest value of stable soil aggregates. No relationship was found between the dry weight of grass roots and the percentage of stable aggregate > .1 mm.

SOME SUBSTITUTED ALKYLAMINO SULFIDES AND SULFONES[1]

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A brief survey of the compounds used in the treatment of malaria and tuberculosis has been given with some discussion of their properties. In order to obtain some compounds which would be of therapeutic value, a large number of compounds containing sulfur, as sulfides and sulfones, has been prepared. The reasons for the use of sulfur-containing compounds as therapeutic agents have been given and the mechanisms of some of the reactions discussed.

A series of sulfur derivatives of chalcones was prepared by reacting diethylaminoalkyl mercaptans and thiols with various substituted chalcones. The condensations involving the dialkylaminoalkyl mercaptans were made by reacting the amine as the hydrochloride. The thiols were condensed under basic conditions. The mechanism of the reaction which is probably 1,4-addition has been discussed. The following compounds were prepared: β-(2-diethylaminoethylmercapto)-β-phenylpropiophenone hydrochloride, m.p. 113–15°; β-(3-diethylaminopropylmercapto)-β-phenylpropiophenone methiodide, m.p. 112–13°; *p*-methoxy-β-(2-diethylaminoethylmercapto)-β-(*p*-dimethylaminophenyl) propiophenone hydrochloride, m.p. 145–6°; *p*-chloro-β-(2-diethylaminoethylmercapto)-β-(*p*-dimethylaminophenyl) propiophenone hydrochloride, m.p. 142–3°; 4-dimethylamino-4′-acetaminochalcone, m.p. 202–3°; *p*-acetamino-β-(2-diethylaminoethylmercapto)-β-(*p*-dimethylaminophenyl) propiophenone hydrochloride, m.p. 153–4°; 2-chloro-4′-acetaminochalcone, m.p. 167°; *p*-acetamino-β-(*p*-tolylmercapto)-β-(o-chlorophenyl) propiophenone, 148–9°; *p*-acetamino-β-(*p*-tolylmercapto)-β-(*p*-methoxyphenyl) propiophenone, m.p. 130–1°.

The reaction of mercaptans with unsymmetrical epoxides has been reviewed and the mechanism of the reaction discussed. A structure proof has also been made to show that the cleavage of unsymmetrical epoxides with mercaptans in a basic medium results in the formation of the β-hydroxyethyl sulfide. The β-hydroxyethyl sulfides prepared in this manner were patterned after the physiologically active epinephrine and ephedrine. The compounds prepared in this series were methyl β-phenyl-β-hydroxyethyl sulfide, b.p., 127°/0.8 mm.; methyl β-phenyl-β-hydroxyethyl sulfide methiodide, m.p. 138–9° (decomp.); methyl phenacyl sulfide, b.p. 130–2°/0.8 mm.; methyl phenacyl sulfide 2,4-dinitrophenylhydrazone, m.p. 164–5.5°; γ-diethylaminopropyl phenacyl sulfide, b.p. 158–60°/0.8 mm.; γ-diethylaminopropyl phenacyl sulfide 2,4-dinitrophenylhydrazone, m.p. 183–4°; γ-diethylaminopropyl β′-phenyl-β′-hydroxyethyl sulfide, b.p. 149–52°/0.5 mm.; β-diethylaminoethyl β′-phenyl-β′-hydroxyethyl sulfide,

[1] Doctoral thesis No. 814, submitted August 26, 1946.

b.p. 132°/0.5 mm.; γ-(β-diethylaminoethylmercapto)propyl β′-phenyl-β′-hydroxyethyl ether, b.p. 180–2°/1.5 mm.; γ-diethylaminopropyl γ′-diethylamino-β′-hydroxypropyl sulfide, b.p. 131–4°/0.5 mm.; β-diethylaminoethyl γ′-diethylamino-β′-hydroxypropyl sulfide, b.p. 121–2°/0.8 mm.; γ-diethylamino-β-hydroxypropyl phenyl sulfide, b.p. 125–8°/0.5 mm.; γ-diethylamino-β-hydroxypropyl *p*-dimethylaminophenyl sulfide, b.p. 145–7°/0.001 mm.; γ-diethylamino-β-hydroxypropyl *p*-tolyl sulfide, b.p. 137°/0.8 mm.; γ-diethylamino-β-hydroxypropyl *p*-aminophenyl sulfide, b.p. 153°/1 mm.; γ-diethylamino-β-hydroxypropyl *p*-chlorophenyl sulfide, b.p. 149–52°/1.5 mm.; γ-diethylamino-β-hydroxypropyl *p*-tolyl sulfone, b.p. 217°/1 mm.; *p*-aminophenyl β-phenyl-β-hydroxyethyl sulfide, b.p. 185°/0.001 mm.; γ-diethylaminopropyl β′-hydroxy-Δ^3-butenyl sulfide, b.p. 123°/0.8 mm.; β-diethylaminoethyl β′-hydroxy-Δ^3-butenyl sulfide, b.p. 111°/1.5 mm.; *p*-aminophenyl β-hydroxy-Δ^3-butenyl sulfide, b.p. 165–8°/0.8 mm.; β-diethylaminoethyl β′-phenyl-β′-hydroxyethyl ether, b.p. 142–4°/0.5 mm.; β-diethylaminoethyl β′-phenyl-β′-acetoxyethyl ether, b.p. 138–41°/0.5 mm.

A group of quinoline sulfides has been prepared by reacting alkyl mercaptides with chloroquinolines. The following new compounds were prepared: 7-chloro-4-quinolyl 4′-methyl-2′-thiazolyl sulfide hydrochloride, m.p. 169–70°; 7-chloro-4-quinolyl β-hydroxyethyl sulfide, m.p. 113–15°; 7-chloro-4-quinolyl β-chloroethyl sulfide hydrochloride, m.p. 196–7°; 7-chloro-4-quinolyl β-chloroethyl sulfide, m.p. 107–8°; 7-chloro-4-quinolyl β-(γ′-diethylaminopropylmercapto)ethyl sulfide dihydrochloride, m.p. 176–7°; 7-chloro-4-quinolyl β-(4′-methyl-2′-thiazolylmercapto)ethyl sulfide hydrochloride, m.p. 205–8°.

The antimalarial activity of N^1-*p*-chlorophenyl-N^5-isopropylbiguanide suggested an investigation of the physiological activity of some sulfur and oxygen analogs of this compound. Thus the following were prepared: 1-*p*-chlorophenyl-2-thiolmethylpseudothiourea hydroiodide, m.p. 165–7°; 1-*p*-chlorophenyl-5-isopropyldithiobiuret, m.p. 135–7°; 1,5-di(*p*-chlorophenyl)-2-thiolmethylpseudodithiobiuret, m.p. 154–5°; 1,5-di(*p*-chlorophenyl)dithiobiuret, m.p. 168°; 1-*p*-chlorophenyl-2-thiolmethyl-5-phenylpseudomonothiobiuret, m.p. 153–4°; 1-*p*-chlorophenyl-5-phenyl-2-monothiobiuret, m.p. 163–5°; 1-*p*-chlorophenyl-2-thiolmethyl-5-methylpseudomonothiobiuret, m.p. 160–1°; 1-*p*-chlorophenyl-5-methyl-2-monothiobiuret, m.p. 182–3°.

In connection with various studies on the preparation of some pharmacologically active compounds the following new compounds were prepared: γ-(*p*-nitrophenoxy)propyl β-morpholinoethyl sulfone, m.p. 98–9°; γ-(*p*-aminophenoxy)propyl β-morpholinoethyl sulfone, m.p. 117°; γ-(*p*-isopropylaminophenoxy)propyl β-morpholinoethyl sulfone (?), m.p. 106–8°; β-hydroxyethyl 2-pyridyl sulfide picrate, m.p. 113–14°; N-γ-diethylaminopropyl-3-methoxy-6-bromoaniline picrate, m.p. 116–17°; N-γ-diethylaminopropyl-*o*-bromoaniline dihydrochloride, m.p. 171–3°; 2-morpholinoquinoline, m.p. 164°; 6-methoxy-2-morpholinoquinoline, m.p. 102–3°; 9-[*p*-(2′,5′-dimethylpyrryl-1′)phenyl]acridine, m.p. 145–7°; *p*-methoxy-*p*′-acetaminobenzophenone, m.p. 170–1°; *p*-methoxy-*p*′-aminobenzophenone, m.p. 121–2°.

MECHANISMS OF ADDITION OF ORGANOLITHIUM COMPOUNDS TO QUINOLINE AND ISOQUINOLINE[1]

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A general review of the literature concerning the reaction of organometallic compounds with pyridine, quinoline, isoquinoline and acridine has been made. Their relationship to the ammonia system has been indicated and the mechanisms of addition of organolithium compounds to such cyclic ammono aldehyde ethers has been discussed. In the course of these studies, a new reaction, namely the addition of organolithium compounds to examples of a cyclic ammono ketone ether, (2-phenylquinoline), has been indicated. This reaction resulted in the preparation of a new type of compound, namely the 2,2-disubstituted-1,2-dihydroquinolines.

A series of 6-quinolyl and substituted 6-quinolyl nitro-, amino-, and acetamidophenyl sulfides and sulfones has been synthesized and submitted for testing for antimalarial and tuberculocidal activity.

One equivalent of *p*-bromoaniline was reacted with three equivalents of *n*-butyllithium. To the insoluble lithium salt of *p*-aminophenyllithium was added two equivalents of quinoline. Subsequent to hydrolysis, nitrobenzene oxidation of the intermediate dihydro product, and distillation, a glass (70 per cent), b.p. 165°–175° (0.005 mm.), was obtained. The picrate was formed and found to be a mixture. By means of the higher solubility in benzene of the picrate of a secondary product of reaction, the insoluble picrate of 2-(*p*-aminophenyl)-quinoline was obtained in pure form as the residue. Decomposition of the picrate with dilute ammonium hydroxide gave 2-(*p*-aminophenyl)-quinoline, m.p. 138°–138.5°; picrate, m.p. 197°–198°. The analyses for the secondary product of reaction most nearly corresponded to those required for x-butyl-x-(*p*-aminophenyl)-quinoline, m.p. 148°–148.5°; picrate, m.p. 220°–221°. Condensation of 2-(*p*-aminophenyl)-quinoline with salicylaldehyde at 150° gave a quantitative yield of 2-(*p*-salicylideneaminophenyl)-quinoline, m.p. 188°–188.5°.

Addition of phenyllithium to 2-phenylquinoline after refluxing and stirring for twelve hours yielded a glassy distillate, b.p. 155°–165° (0.02–0.03 mm.). The product formed an insoluble hydrochloride (35 per cent) in dilute hydrochloric acid, by which means the compound was separated from the soluble 2-phenylquinoline present in small amount. The hydrochloride was decomposed with 10 per cent sodium hydroxide. Charcoaling and crystallization gave 2,2-diphenyl-1,2-dihydroquinoline, m.p. 86°–87°. To establish the fact that 1,2-addition had occurred, 2-phenyl-2-(*p*-tolyl)-1,2-dihydroquinoline was prepared by the same reaction using different starting materials. Thus, *p*-tolyllithium was added to 2-phenylquinoline,

[1] Doctoral thesis No. 821, submitted December 16, 1946.

and phenyllithium was added to 2-(*p*-tolyl)-quinoline. The products from each reaction, namely 2-phenyl-2-(*p*-tolyl)-1,2-dihydroquinoline, melted at 86°–87° and were identical (mixed m.p.).

2-(*o*-Biphenylyl)-quinoline (50 per cent) was prepared by addition of *o*-biphenyllithium to quinoline; b.p. 162°–166° (0.007 mm.), m.p. 102°–103°. 2-(*p*-Biphenylyl)-quinoline, m.p. 178°–179°, was prepared by the reaction of *p*-biphenylyllithium with quinoline.

5-Aminoisoquinoline and an excess of acetonylacetone when refluxed two hours, gave 5-(2,5-dimethyl-1-pyrryl)-isoquinoline (95 per cent), m.p. 83°–84°; picrate, m.p. 174°–175°. Condensation of 4-aminoisoquinoline and acetonylacetone by refluxing three hours gave 4-(2,5-dimethyl-1-pyrryl)-isoquinoline (97 per cent), m.p. 77°–78°. 6-Aminoquinoline, when condensed with acetonylacetone gave 6-(2,5-dimethyl-1-pyrryl)-quinoline (93 per cent), m.p. 98°–99°; picrate, m.p. 185°–186°.

Addition of *p*-dimethylaminophenyllithium to isoquinoline in ether gave, subsequent to hydrolysis with water, nitrobenzene oxidation, and distillation, 1-(*p*-dimethylaminophenyl)-isoquinoline (55 per cent), b.p. 196°–199° (4 mm.), m.p. 114.5°–115°; picrate, m.p. 220°–221°. By the same procedure *p*-tolyllithium and isoquinoline gave 1-(*p*-tolyl)-isoquinoline (55 per cent), b.p. 174°–183° (5 mm.); m.p. 71°–72°. Similar addition of *p*-(2,5-dimethyl-1-pyrryl)-phenyllithium to isoquinoline yielded 1-[*p*-(2,5-dimethyl-1-pyrryl)-phenyl]-isoquinoline (45 per cent), b.p. 203°–208° (4 mm.); m.p. 159°–160°. 1-(*p*-Anisyl)-5-(2,5-dimethyl-1-pyrryl)-isoquinoline (38 per cent), b.p. 222°–228° (2 mm.), was obtained from the interaction of *p*-anisyllithium and 5-(2,5-dimethyl-1-pyrryl)-isoquinoline. From the reaction between 5-(2,5-dimethyl-1-pyrryl)-isoquinoline and *p*-(2,5-dimethyl-1-pyrryl)-phenyllithium, 1-[*p*-(2,5-dimethyl-1-pyrryl)-phenyl]-5-(2,5-dimethyl-1-pyrryl)-isoquinoline (31 per cent), b.p. 220°–230° (0.05 mm.); m.p. 214°–215° was obtained.

1-(*p*-Nitrophenyl)-isoquinoline (60 per cent), m.p. 155°–156°, was prepared by dehydrogenation of 1-(*p*-nitrophenyl)-3,4-dihydroisoquinoline, with use of palladium black. 1-(*p*-Aminophenyl)-isoquinoline (70 per cent), m.p. 191°–192°, was prepared by reduction of 1-(*p*-nitrophenyl)-isoquinoline with hydrogen in the presence of Raney nickel. The same compound (mixed m.p.) was prepared (70 per cent) by the addition of the lithium salt of *p*-aminophenyllithium (obtained by halogen-metal interconversion of *p*-bromoaniline with *n*-butyllithium) to isoquinoline as described under 2-(*p*-aminophenyl)-quinoline. By the same means, 1-(*p*-mercaptophenyl)-isoquinoline hydrochloride was prepared by the addition of the lithium salt of *p*-mercaptophenyllithium to isoquinoline. Subsequent to hydrolysis, alkaline extraction, acidification with acetic acid and ether extraction, the hydrochloride (20 per cent) was precipitated with ethereal hydrogen chloride, m.p. 271°–272°. Carbonation of an aliquot of the lithium salt of *p*-mercaptophenyllithium gave *p*-mercaptobenzoic acid (75 per cent), m.p. 214°–215°.

6-Quinolyl *p*-nitrophenyl sulfone (67 per cent), m.p. 181°–182°, was prepared by the Skraup synthesis applied to 4-nitro-4′-aminodiphenyl

sulfone (or the acetamido compound) using the arsenic oxide modification.[2] Reduction with hydrogen (Raney nickel catalyst) gave 6-quinolyl *p*-aminophenyl sulfone (88 per cent), m.p. 178°–179°. A similar Skraup synthesis gave 6-quinolyl *p*-nitrophenyl sulfide (55 per cent), m.p. 168.5°–169.5°. 6-Quinolyl *p*-aminophenyl sulfide dihydrochloride (78 per cent), m.p. 217°–218°, was prepared by reduction of the latter as given above, and the product was converted to the hydrochloride with ethanolic hydrogen chloride.

p-Aminothiophenol (69 per cent), b.p. 143°–146° (17 mm.), m.p. 43°–45°, was prepared by the interaction of *p*-chloronitrobenzene and aqueous sodium sulfide. 5-Nitro-6-chloroquinoline and the sodium salt of *p*-aminothiophenol gave 5-nitro-6-quinolyl *p*-aminophenyl sulfide (90 per cent), m.p. 137°–138°. Reduction with hydrogen in the presence of Raney nickel, and conversion to the hydrochloride gave 5-amino-6-quinolyl *p*-aminophenyl sulfide trihydrochloride (88 per cent), m.p. 261°–262°. 5-Nitro-6-chloroquinoline and the sodium salt of *p*-acetamidothiophenol gave 5-nitro-6-quinolyl *p*-acetamidophenyl sulfide (80 per cent), m.p. 173°–174°. The compound was also obtained by acetylation of 5-nitro-6-quinolyl *p*-aminophenyl sulfide. Condensation of the sodium salt of *p*-chlorothiophenol and 5-nitro-6-chloroquinoline gave 5-nitro-6-quinolyl *p*-chlorophenyl sulfide (88 per cent), m.p. 115°–116°.

From the reaction of the sodium salt of *p*-acetamidobenzenesulfinic acid and 5-nitro-6-chloroquinoline, 5-nitro-6-quinolyl *p*-acetamidophenyl sulfone (quantitative yield), m.p. 247°–248°, was obtained. Deacetylation with boiling dilute hydrochloric acid gave 5-nitro-6-quinolyl *p*-aminophenyl sulfone (quantitative yield), m.p. 258°–259°. 5-Nitro-8-acetamido-6-quinolyl *p*-acetamidophenyl sulfone (92 per cent), m.p. 313°–314°, resulted from the reaction of 5-nitro-6-chloro-8-acetamidoquinoline[3] and sodium *p*-acetamidobenzenesulfinate. Deacetylation with boiling dilute hydrochloric acid and neutralization gave 5-nitro-8-amino-6-quinolyl *p*-aminophenyl sulfone (91 per cent), m.p. 247°–247.5°. Condensation of 5-nitro-6-chloro-8-aminoquinoline[3] with sodium *p*-acetamidobenzenesulfinate gave 5-nitro-8-amino-6-quinolyl *p*-acetamidophenyl sulfone (95 per cent), m.p. 261.5°–262.5°.

Oxidation of γ-(*p*-nitrophenoxy)-propyl β-hydroxyethyl sulfide[4] by hydrogen peroxide in glacial acetic acid medium yielded the corresponding sulfone (85 per cent), m.p. 103°–104°, together with a small amount of the acetoxy compound, m.p. 85°–86°. The crude sulfone was therefore refluxed with 2N sulfuric acid to hydrolyse the ester. The acetoxy compound was prepared (quantitative yield) either by acetylation of the pure γ-(*p*-nitrophenoxy)-propyl β-hydroxyethyl sulfone or by acetylation of the sulfide previous to oxidation (80 per cent). Reduction of the γ-(*p*-nitrophenoxy)-propyl β-hydroxyethyl sulfone with hydrogen (Raney

[2] Richter and Smith, Jour. Amer. Chem. Soc., 66, 397 (1944).
[3] Gilman, Benkeser, Gainer, Lindblad, Marshall, Massie, Myers, and Tolman, Jour. Amer. Chem. Soc., 68, 1577 (1946).
[4] Gilman and Fullhart, Jour. Amer. Chem. Soc., 67, 1585 (1945).

nickel) gave γ-(*p*-aminophenoxy)- propyl β-hydroxyethyl sulfone (92 per cent), m.p. 133.5°–134°. The latter compound, when added to ice-cold thionyl chloride, gave, on warming and subsequent decomposition of excess thionyl chloride with absolute ethanol, γ-(*p*-aminophenoxy)-propyl β-chloroethyl sulfone hydrochloride (78 per cent), m.p. 179°–180°.

Condensation of 6-methoxy-8-aminoquinoline with γ-chloropropyl mercaptan at 130°–135° for sixteen hours gave [subsequent to acid extraction, basic extraction (sodium hydroxide), acidification, neutralization (ammonium hydroxide) and ether extraction, followed by distillation] an oil (32 per cent), b.p. 174°–178° (0.5 mm.); hydrochloride, m.p. 172°–173.5°.

From the reaction of 4-bromoisoquinoline and sodium hydroxide in the presence of copper bronze powder and copper sulfate, heated to 210° for twelve hours in a bomb chamber, 4-hydroxyisoquinoline was obtained (61 per cent), m.p. 223°; picrate, m.p. 243°–244°.

FIELD INVESTIGATION OF CALCIUM ARSENATE, LEAD ARSENATE, AND SPREADING AGENTS FOR CODLING MOTH SPRAYS, AND AN ABRIDGED FORM OF CHI-SQUARE[1]

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When this investigation was undertaken (1939) the predominate codling moth insecticide was lead arsenate. An attempt was made to find a substitute which was cheaper and did not leave as toxic a residue on apples. As calcium arsenate seemed to offer fair promise of effectiveness in Iowa, it received first consideration as a substitute. Calcium arsenate was compared directly with lead arsenate in a commercial apple orchard near Mitchellville, Iowa. The experimental design consisted of 4 single row plots in each of 4 randomized blocks, which was duplicated in another section of the orchard. Two plots of each block were sprayed with calcium arsenate mixtures; the other two with lead arsenate mixtures. Two hundred apples from each of 6 randomly selected count trees were examined for codling moth injury 3 times during the season. The last count was made just prior to harvest.

The results of this experiment, augmented by the work of other experimenters, indicated that, in general, lead arsenate was superior to calcium arsenate as an insecticide for the codling moth. Calcium arsenate, however, could generally be depended upon for controlling the first brood codling moth in Iowa. The use of lead arsenate was indicated for heavy first brood infestations and for moderate to heavy second brood infestations.

As the use of spreading agents in codling moth sprays was questionable they were included in the investigation. In 1939 soybean flour was tested. In 1940 soybean flour, lime-casein and a sodium oleyl sulfate-resin type of spreader-sticker were tested. Conclusive results for soybean flour and lime-casein were obtained in that neither affected the efficacy of lead arsenate as a codling moth insecticide under Iowa conditions. Although no advantage was detected in this test, some advantage in the use of the sodium oleyl sulfate-resin spreader-sticker might be shown by more extensive studies. It can be concluded that the improvement of the physical properties of a spray mixture is not necessarily accompanied by increased insecticidal effect.

In evaluating the effectiveness of insecticides the investigator has need of a criterion of effect. Several different criteria have been used in the evaluation of codling moth damage to apples, but little was known concerning their relative merits. In order to increase the information

[1] Doctoral thesis No. 841, submitted May 26, 1947.

known about this subject it was included in the investigation. In the 1939 season 40 apples from each of 6 trees of each plot were cut open and examined for the number of injured fruit, worms, stings and injuries (worms + stings). In the 1940 season 25 apples from each of 4 trees of each plot of the section containing uniform trees were examined for wormy fruit, stung fruit, injured fruit, worms, stings and injuries (worms + stings). The results of these examinations indicated that satisfactory comparisons of different treatments in field experiments could be made on the basis of the number of injured fruit or the number of wormy fruit. Other criteria would be useful in making more detailed studies. An index number based on the use of the ratio of worms to stings was also tested and proved to be unreliable.

The relationship between the number of injuries and the number of injured fruit and between the number of worms and the number of wormy fruit can be expressed by the general equation

$$Y = aX^b.$$

Using the 1939 data the regression of the number of injuries per injured fruit was found to be

$$Y = 1.555X^{0.9942}.$$

From the 1940 data the number of injuries per injured fruit was expressed by

$$Y = 0.0105X^{2.9492}.$$

From the 1940 data the number of worms per wormy fruit was computed to be

$$Y = 1.112X^{1.2694}.$$

Hansberry obtained

$$Y = 1.049X^{1.1096}$$

for the equation of the number of worms per 100 apples based on percentage of wormy apples from data taken at Wenatchee, Washington in 1932 and 1933. These equations can be useful in converting data from one criterion to another, but they differ with season and locality which must be considered when they are employed.

The chi-square equation is useful in testing differences in laboratory comparisons of insecticides. The time consuming calculations involved are a drawback to its use in extensive studies. A simplified equation, based on the fourfold table, for determining the value of chi-square when there is one degree of freedom and the sample size is constant follows:

$$X^2 = \frac{2k\,(a - c)^2}{(a + c)\,(b + d)}.$$

Its use is limited by the requirement of a constant sample size, but in

laboratory experiments the samples are usually of uniform size. In order to incorporate Yates' correction for continuity the equation is changed to

$$X^2 = \frac{2k(|a-c|-1)^2}{(a+c)(b+d)}.$$

The first of these two equations can be employed in constructing a table of fiducial limits. If the terms in it are rearranged with respect to c the following is formed:

$$(2k + X^2)c^2 - 2(2ka + bX^2)c + a[a(2k + X^2) - 2kX^2] = 0.$$

When $P = 0.05$ the value of $X^2 = 3.841$. This value can be substituted for X^2 to obtain

$$(2k + 3.841)c^2 - 2(2ka + 3.841b)c + a[a(2k + 3.841) - 7.682k] = 0$$

which is in the form of the general quadratic equation. If values are substituted for a, two values for c can be determined for each substitution. These values will form the limits of significance when $P = 0.05$. For Yates' correction for continuity to be included the values of a that are substituted should vary by 0.5. The values that are then obtained will have to be changed by 0.5 to obtain the proper fiducial limits. These limits can then be arranged in tabular form. For the proper functioning of such a table the sample size must be constant.

ECOLOGICAL FACTORS AFFECTING THE ACTIVITY OF THE WESTERN FOX SQUIRREL, *SCIURUS NIGER RUFIVENTER* (GEOFFROY)[1]

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The purpose of this investigation was to measure certain attributes of weather and time, and to determine if they have any effects upon the fox squirrel by using the degree of its activity as a gauge of effects. By associating these effects with those caused by biological agents and other physical factors, a more thorough understanding of the ecology and management of the fox squirrel can be obtained.

The area chosen for the investigation consists of approximately 250 acres located along Squaw Creek, its tributary, Onion Creek, and the vicinity nearby in Sections 29, 32 and 33 of Franklin Township, Story County, Iowa. Floodplain and upland are the major topographic types.

Measurement of squirrel activity was obtained by using two count methods. The linear count consists of recording the number of squirrels seen as the worker walks a course of estimated length and width. The spot count or time-area count consists of recording the number of squirrels seen as the worker remains at a certain spot for a definite length of time. The following data were recorded during each count attempt for either of the two count methods: date, time of day in the field, degree of overcast, number of squirrels seen, time at which squirrels were seen, locality (ridge, ravine, floodplain) on which squirrels were seen, distance walked during observations, acreage observed, per cent of defoliation, count method used, and supplementary remarks such as presence and depth of snow, presence of precipitation (rain, snow, sleet, hail, mist, fog), foraging and feeding activity especially in cultivated tracts, mating actions, nest-building activity, play and animality traits. Readings of temperature, relative humidity, barometric pressure, precipitation and wind velocity were obtained for each count attempt.

The data for each ecological factor were analyzed separately to reveal whatever effects it might have upon squirrel activity. At the same time, cognizance was taken of the complex coactions exhibited by two or more ecological factors operating simultaneously.

Magnitude of fox squirrel activity in Iowa fluctuates with the seasons. Activity is greater in autumn than in any other season, and it is least in spring and summer. December shows the most intense activity and July has the least.

The fox squirrel is diurnal. It is seldom active before sunrise or after sunset. By hourly segregation most activity occurs from 7:00 A.M.

[1] Doctoral thesis No. 845, submitted June 6, 1947.

to 8:00 A.M. Least daytime activity occurs from 2:00 P.M. to 5:00 P.M. There are three peaks of activity throughout the day. The first is from 6:00 A.M. to 9:00 A.M. The second is from 11:00 A.M. to 1:00 P.M. The third and minor peak is from 6:00 P.M. to 7:00 P.M.

Activity in winter is greatest from 8:00 A.M. to 9:00 A.M. and from 12:00 M. to 2:00 P.M. It is least from 2:00 P.M. to 5:00 P.M. Spring activity is greatest from 6:00 A.M. to 11:00 A.M. and is least from 2:00 P.M. to 4:00 P.M. Greatest activity in summer occurs from 6:00 A.M. to 7:00 A.M. and from 6:00 P.M. to 7:00 P.M. Least activity occurs from 4:00 A.M. to 6:00 A.M., from 3:00 P.M. to 5:00 P.M. and from 7:00 P.M. to 8:00 P.M. Activity in autumn is most intense from 6:00 A.M. to 9:00 A.M. and from 11:00 A.M. to 12:00 M. It is least from 5:00 A.M. to 6:00 A.M. and from 5:00 P.M. to 6:00 P.M.

Seasonal variations of activity intensity during a day are the results of interactions of several factors. Excessive summer heat, welcome winter heat, and plentiful food supplies in autumn are important examples.

The fox squirrel is most active at temperatures ranging between forty degrees Fahrenheit and forty-nine degrees Fahrenheit. Temperatures at which activity is least are those from fifteen to nineteen degrees Fahrenheit and from seventy to eighty-four degrees Fahrenheit.

Data indicate that the fox squirrel is active in all temperatures of natural environment. However, at temperatures of ninety degrees Fahrenheit and above, it does display unmistakable lethargy. Under such temperature conditions the fox squirrel appears inclined to exert itself as little as possible. Extremely low temperatures by themselves do not retard squirrel activity as much as do high temperatures. However, low temperatures associated with other meteorological factors such as high wind, complete overcast or driving snow restrict movement very noticeably.

Fox squirrel activity is not dependent solely upon temperature fluctuations. In several instances consecutive mean hourly temperatures of approximately equal value were not accompanied by respective similar amounts of activity.

Greatest activity occurs at relative humidities between fifty and fifty-nine per cent and between eighty and ninety-nine per cent. Least activity occurs at relative humidities from twenty per cent to thirty-nine per cent. It is not known to what extent relative humidity *per se* modifies squirrel activity. Much activity occurs in early morning and early forenoon when relative humidities are often high. However, time of day and temperature as well as humidity are comparably important influences.

Wind velocity is one of the most influential environmental factors affecting squirrel activity. Magnitude of activity is inversely proportional to wind velocity. Of all environmental influences considered, the effects of wind are least interrelated with the effects of other factors.

A high wind does not restrict squirrel activity on the ground as much as it restricts movement in trees. The force of a strong wind against

a squirrel's body and especially against its tail increases the animal's difficulty in maintaining balance while it is in the process of leaping and when it is traversing small branches.

Specific effects of barometric pressure upon squirrel activity are non-evident. Activity is greatest from 30.40 inches to 30.49 inches and from 30.10 inches to 30.29 inches at 800 feet above sea level. Least activity occurs at pressures between 29.60 inches and 29.69 inches.

Intensity of activity is inversely proportional to the degree of cloudiness. Least activity occurs on cloudy days. Greatest activity occurs on clear days. Complete absence of sunshine decreases squirrel activity. Partial sunshine, if only for a brief portion of an observation period, stimulates activity.

Light rains, drizzles and mists are relatively moderate in their effects upon squirrel activity. Snowfall decreases activity but to a lesser extent than does rain. The fox squirrel is more active at those times which have no precipitation than at those which have rain or snow. The effect of fallen snow upon squirrel activity depends upon depth of the snow. Activity is unaffected by snow that is two inches or less deep. Snow more than two inches deep impedes activity. Snow patches have no appreciable effect upon activity.

The effects of each environmental factor must be interpreted only after due consideration has been given to the interrelationships of that specific factor with the other influences comprising the environment.

BIOLOGY AND CONTROL OF THE TUBER FLEA BEETLE, *EPITRIX TUBERIS* GENTNER, IN NEBRASKA[1]

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The tuber flea beetle, *Epitrix tuberis* Gentner, is the most important of the insect pests found on potatoes in western Nebraska, especially in the irrigated sections of the North Platte Valley. This species, which until recently was considered the same as the potato flea beetle, *E. cucumeris* (Harris), is western in distribution, apparently reaching the eastern limits of its range in Nebraska. Tuber blemishes caused by the feeding activities of the larvae are major factors in lowering the quality of the potato crop.

Tuber flea beetles overwinter in the soil in the adult stage, relatively high average winter survivals being recorded in cage studies. Following spring emergence the adults feed on the foliage of a wide range of plants; 28 different species were recorded as adult hosts. Whenever potatoes were available, they were preferred.

Insectary studies indicated an average preoviposition period of between five and six days; an oviposition period of from 35 to 57 days, averaging 44.7 days; and the number of eggs laid per female ranged from 161 to 215, averaging 187. Eggs usually were deposited in batches of from 12 to 15 with intervals of from 1 to 2 days elapsing between successive depositions.

The average duration of the egg stage ranged from 5.5 to 6.2 days, the larval period from 17.7 to 22.8 days and the average length of the pupal stage varied from 5.8 to 11.2 days. Theoretical total developmental periods of from 27 to 85 days were indicated from this study.

In the Scottsbluff area adults begin emerging from the soil about mid-May and continue to appear until late June or early July. The peak of emergence normally is reached shortly before mid-June. Soon thereafter overwintered beetles move into early planted potato fields. Eggs are laid in the soil around the base of the plants from late May until at least the latter part of July. About July 1, first generation adults begin appearing and attain greatest numbers in early fields during the latter half of July or in early August. During July and early August, when the early crop matures or is harvested, the beetles migrate to nearby late plantings. Here eggs are laid and from these develop the second generation larvae which largely are responsible for tuber injury to the late crop. The first adults of the second generation usually appear about August 10 and reach a peak of abundance sometime between late August and mid-September. In the North Platte Valley two fairly complete generations develop each

[1] Doctoral thesis No. 818, submitted December 4, 1946.

year, and under some conditions evidence of a partial third has been obtained.

Larval feeding is confined to the underground portions of the plant. "Worm tracks," "pimples" and "slivers" are caused by the larvae feeding on tubers of the potato plant. In Nebraska these blemishes, resulting in low quality potatoes, are far more serious than foliage injury by the adults. Larvae were found to develop on seven different host plants.

Factors of most importance in influencing the abundance of tuber flea beetles are weather conditions, especially rainfall, irrigation, host plants, date of planting the potato crop and the presence or absence of cull dumps.

From data presented it is evident that seasons with above normal rainfall are most favorable for flea beetle development and subsequent tuber injury. Adequate moisture during September and October and the following May and June favors winter survival and spring emergence of the overwintered adults. A comparison of tubers from plots of potatoes receiving five or six irrigations during the season showed almost three times as many larval blemishes as potatoes irrigated twice and nearly eight times the number found on tubers from plots watered once.

The type of food plant consumed by adult flea beetles markedly influenced oviposition and length of life. Potato foliage was the most satisfactory of the nine host plants studied, egg production being highest and mortality lowest on such a diet. The substitution of potato foliage for less nutritious diets was followed in two or three days by increased egg production. A change from potato to foliage of other food plants resulted in decreased egg production. In one 60-day test significant fluctuations in egg deposition were recorded when, at 10-day intervals, diets of potato foliage were alternated with either marsh elder, *Iva xanthifolia*, or buffalo bur, *Solanum rostratum*.

In the field, larvae developed most readily and in largest numbers on potato roots and tubers. Considerably less development occurred on roots of tomato. Relatively few individuals matured on ground cherry, *Physalis lanceolata*, black nightshade, *Solanum nigrum*, buffalo bur and field bean, *Phaseolus vulgaris*. No larval development was recorded from wild tomato, *S. triflorum*, marsh elder or kochia, *Kochia scoparia*.

As a result of these findings and since largest populations of tuber flea beetles were found to occur in localities where early planted potatoes are grown, the elimination of the relatively few such plantings was proposed. The data indicated that the elimination of early plantings would lower the general flea beetle population level by reducing the reproductive capacity and longevity of the overwintered beetles. Without potatoes the overwintered insects would be forced to feed, live and reproduce on less nutritious host plants, thus greatly decreasing the number of first generation beetles available to infest late planted potato fields.

Not only do the larvae reduce the quality of the potato crop but data are presented to show that they also are responsible for a portion of the low total yields often obtained in early planted fields. Approximately

30 per cent of the stolons, or potential tuber-forming parts, were destroyed by the feeding activities of the larvae.

In a varietal susceptibility test involving eight Nebraska adapted varieties, the Bliss Triumph, most widely grown potato in western Nebraska, proved most resistant to larval injury.

Dusts, properly applied, equalled sprays in effectiveness for the control of tuber flea beetles. Of the dusts tested in the earlier experiments (1940–1943), barium fluosilicate and cryolite, which were equally effective, ranked first. Among the sprays used in these early experiments, zinc arsenite and basic copper arsenate proved best. In the 1944 field and insectary experiments, DDT in both spray and dust form was significantly superior to all other materials for controlling flea beetles. DDT-sulfur dusts appeared slightly more efficient than DDT-pyrophyllite mixtures in 1945 tests. To effectively control flea beetles in infested late planted fields three or more treatments were needed. Single applications proved unsatisfactory. Applications made in August or just before the vines closed the rows were of greatest value in the control program.

In a preliminary test, soil applications of DDT-pyrophyllite dusts significantly reduced the amount of larval injury to tubers.

No increases in aphids or other harmful species have occurred following the use of DDT on potatoes in western Nebraska. Although some beneficial insect species were killed with the DDT insecticides, large scale tests indicated that this would have no appreciable effect on the insect problem as a whole.

The cultural practices found effective as control measures for tuber flea beetles also aid in the control of most other pests of the potato in Nebraska.

WATER CONTENT CHANGES OF POULTRY HELD IN FROZEN STORAGE AS RELATED TO PALATABILITY[1]

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The purpose of this investigation was to explore the nature of water content changes of poultry held in frozen storage and to study the relationship of these changes to juiciness scores.

Vapor pressure analyses for bound water and total water content were made on the pectoralis major breast muscles of 60 roasters. These analyses were made first on the fresh raw muscle, later after frozen storage, and finally after cooking.

The roasters were divided for frozen storage into series A, B, and C, each series containing 20 birds. Each series was then subdivided into two groups, one group of 10 birds being stored at —12.2°C. (10°F.) and the other 10 birds being stored at —23.3°C. (—10°F.). Series A was stored for 9 months, series B for 6 months, and series C for 9 months. However, series C differed from series A in that samples for vapor pressure analyses were removed from the roasters at the end of 6 months of storage as well as at the end of the 9-month period.

The experimental apparatus used was an isotenoscope type of instrument. Duplicates of samples of the breast muscle were dehydrated at 27.5°C. (81.5°F.) under a high vacuum. Vapor pressure desorption isotherms were determined for each group of 10 birds in each series (A, B, and C). These isotherms were further analyzed statistically in order to determine absolute differences between them. Only the upper 60 per cent of the R. V. P. range was considered in the comparison analysis.

The vapor pressure method of analysis successfully indicated an increase in bound water in chicken breast muscle during storage and also a further increase in waterbinding capacity as a result of cooking. The longer storage time (9 months) and the higher storage temperature (—12.2°C.) apparently caused the greater amount of bound water.

The average water content of all of the fresh samples was found to be 74.4 per cent. The free water lost because of storage was slight, but that lost because of cooking was 4.1 per cent.

No correlation was found between juiciness and either bound water or total per cent water in the breast muscle of the roasters. However, further study with an improved palatability scoring plan and a different experimental plan might indicate otherwise. It may be noted, however, that slight correlation trends between bound water and palatability and between total moisture content and palatability did appear in the results.

[1] Doctoral thesis No. 804, submitted July 20, 1946.

Worthy of note is the fact that the vapor pressure of water, as indicated by the experimental apparatus used, varied according to room temperature. It was, therefore, necessary to make corrections experimentally for the vapor pressure readings of the roaster breast muscles.

Equilibrium in the vapor pressure apparatus when making tests was at times difficult to obtain. This was usually true of the cooked samples and occasionally it was true with the uncooked samples. Cooking seemed to complicate the dehydration process and the establishment of vapor pressure equilibrium.

This work seemed to give evidence that in poultry breast muscle protein denaturation continues during frozen storage, being more for the longer storage time, and also for the higher storage temperature. It also offers some experimental evidence that muscle myosin changes from an elongated-shaped molecule to a more rolled-up type of molecule during the denaturation process.

THE GRADUATED LAND TAX[1]

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The graduated land tax is a progressive tax on land, that is, a tax whose rate increases as the size of the tax base, above specified exemptions, also increases. It may be levied either as a supplemental source of fiscal revenue or as a means of exercising social control over the ownership of land. It is with this latter alternative that this study is concerned.

The specific function of the graduated land tax is to preserve for individuals the opportunity of acquiring land through a limitation on the land which may be owned by any one person. Such a regulatory tax clearly involves the use of the Police Power. The determinants lie in the fields of ethics and politics as well as in economics. Given a condition where access to property is prohibited or impeded by "excessive" land holdings, this study has favored the limitation in the interest of the general welfare. It has also been argued that the limitation would probably be upheld as a valid exercise of the Police Power.

This is the ethico-political foundation of the graduated land tax. The politico-economic problem of the tax is not a matter of principle, but a problem in the *application* of the Police Power. It is in the application of any principle that the method of trial and error, study, and, above all, flexibility to meet the dynamic character of the problem, should prevail. There is no final answer in the application of principle, but in this case, the application of the graduated land tax should be dependent on the evidence of sufficient cause.

The First Legislature of the State of Oklahoma adopted a graduated land tax law in 1908. The law was held unconstitutional by the Oklahoma Supreme Court on the grounds that the Act failed to specify the purpose and disposition of the tax revenue. A second graduated land tax was adopted by the Oklahoma Legislature in 1913. This law has been inferred unconstitutional because of the Oklahoma Supreme Court's ruling on the general revenue section of the Act of which it was a part. The most recent Oklahoma proposal for a graduated land tax came with Initiative Petition No. 145, which was circulated in 1935 and was voted on as a State Question in the general election of 1940. This State Question, although receiving a two to one affirmative margin of the 600,000 votes cast, was rejected because of a constitutional requirement that a State Question must carry a majority in terms of the largest vote on any office in the election. In effect, the silent voters on the Question killed it. The unusual feature of the Oklahoma experience with graduated land tax legislation and pro-

[1] Doctoral thesis No. 854, submitted June 9, 1947.

posals is that, in no instance, has the fundamental principle—that of progressive land taxation—been judicially considered.

Using the existing data relating to the ownership of land in Oklahoma, the provisions of the constitutional amendment proposed in Initiative Petition No. 145 were analyzed. This proposal dealt with land holdings exceeding 640 acres. It was established that in 1935, 2.4 per cent of the State's land owners owned more than 640 acres of land and these holdings amounted to 20.2 per cent of the State's land area. The bulk of these holdings were in the timber land and the grazing land areas of the State.

An initial exemption of $12,000 assessed value eliminated 49 per cent of the above land holdings. Exemption of timber land and grazing land eliminated another 36 per cent of the large land holdings. Exemption of land acquired by corporations through mortgage foreclosure eliminated another 8 per cent of the land originally classified. There remained subject to the tax but 7 per cent of the original large land holdings embracing roughly 1.5 per cent of the State's land area.

Within the holdings subject to the tax, the excess acreage above the proposed legal maximum amounted to nearly 244,000 acres, or roughly 0.54 per cent of the State's land area. Of the holdings subject to the tax, 78.5 per cent had less than 640 acres excess and these holdings accounted for 43.7 per cent of the excess acreage. It was further estimated that 70.9 per cent of the taxable holdings were owner-operated and that these holdings accounted for 40.2 per cent of the excess acreage. Where the holding was tenant-operated, disposal of excess acreage would have been economically feasible. In the case of the owner-operator, the decision would have been one of weighing loss in income from reduced operations plus investment income against the amount of the tax.

A suggested law was proposed based upon the analysis of Initiative Petition No. 145 and the experience of foreign graduated land taxes. The proposed law would function under a permissive constitutional amendment. Its tax base was predicated upon assessed valuation. An initial exemption of $25,000 was proposed with an "in lieu" exemption of 320 acres. On the basis of property classification exemptions were also proposed applying to (1) the land of common carriers and public utilities (2) land acquired through collection of debts: this exemption would be limited to seven years (3) improvements on land up to 20 per cent of the assessed value of such land. The proposed rate schedule was composed of 16 rate classes, graduated at intervals of $2,000 assessed value. The rates were to be increased by their original amount each year for the first five years, after which time they would remain at the highest rate reached.

This proposed law would be effective in breaking up excessive holdings. It would be, however, a law which would have little effect upon the present Oklahoma land ownership pattern. Essentially, it would be a preventive—not a remedial—law.

On the basis of the present study, it would seem that the pattern of land ownership existing in Oklahoma in 1935 did not represent an owner-

ship concentration which would constitute a serious impediment to the acquisition of land. Evidence indicates that the graduated land tax proposal embodied in the Oklahoma Initiative Petition No. 145 would have had little incidence upon the 1935 land ownership pattern. It would appear that a more efficient graduated land tax law could be framed for Oklahoma in terms of preventing a potential concentration of land ownership rather than in terms of remedying the then existing land ownership concentration. This study reveals the need for careful study and analysis preceding the enactment of any graduated land tax legislation. The ends of social justice and economic efficiency require that any such legislation represent a maximum flexibility in terms of the dynamic problem that the graduated land tax seeks to solve.

EVALUATION OF CERTAIN INSECTICIDES AND DRUGS AS CHEMOTHERAPEUTIC AGENTS FOR EXTERNAL BLOOD SUCKING PARASITES[1]

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In 1944 Lindquist, Knipling, Jones, and Madden (J. Econ. Ent. 37 (1): 128) demonstrated that bedbugs (*Cimex lectularius* L. and *C. hemipterus* F.) were killed when they engorged on rabbits which had been given, orally, high dosages (300 to 500 mg. per kg.) of DDT or pyrethrins. This was the first demonstration that blood sucking arthropods could be destroyed with chemotherapeutic agents.

More extensive investigations in the field of chemotherapy for insect control were undertaken by the writer in 1946, at the Orlando, Florida, laboratory of the Bureau of Entomology and Plant Quarantine. More than 30 selected insecticides and drugs were evaluated when administered to rabbits. These included well-known materials such as pyrethrins, DDT, rotenone, chlorinated camphene (Hercules 3956), chlordane (Velsicol 1068), several indandione derivatives, phenothiazine, the gamma isomer of benzene hexachloride, atabrine, and neoarsphenamine. The value of the chemicals as therapeutic agents was determined by permitting body lice (*Pediculus humanus corporis* Deg.) and mosquitoes (*Aedes aegypti* L.) to engorge on the treated animals. The effect of several materials was determined also for the ear mite *Psoroptes equi* var. *cuniculi* Del. and the tick *Amblyomma americanum* L.

Initial tests were conducted at the relatively high dosage rate of 300 mg. of the chemical per kg. of body weight of the host. The chemicals, undiluted, were administered in gelatin capsules. A stomach tube was employed when they were administered in oil solution. From 25 to 50 lice, and usually from 15 to 50 mosquitoes, were permitted to engorge 6 hours after administration of the chemical to the host. Tests were made also at 24 and usually at 48 hours after the chemical was given to the rabbits. Thereafter, tests were not made unless the mortality records indicated that the chemical was producing some toxic action to the insects. The engorged insects were held at 85°F. and mortality counts were made after 24 hours.

Of thirty-one chemicals evaluated against mosquitoes, several showed some activity but only one, the gamma isomer of benzene hexachloride,

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[1] Doctoral thesis No. 838, submitted March 19, 1947.

[2] The studies herein reported were undertaken by the author while engaged in regular research activities sponsored and financed by the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Details of the investigations will be published at some future date.

caused complete mortality of the insects at the initial dosage of 300 mg. per kg. of body weight of the host. Subsequent tests employing lower dosage levels showed that complete kill of mosquitoes resulted also at 25 and 100 mg. per kg. Most of the animals were killed by the chemical. However, one receiving 100 mg. per kg. survived, and the mosquitoes engorging each day on this host showed complete kill for 5 days. A dosage of 5 mg. per kg. caused a maximum of 93 per cent kill of engorged mosquitoes and caused no apparent harm to the host.

Thirty-three chemicals were evaluated against the body louse. Several of the materials showed activity but only chemicals among the indandione derivatives caused complete mortality at the initial dosage of 300 mg. per kg. One of these, 2-pivalyl-1,3-indandione, proved especially active and subsequent tests showed that oral dosages as low as 2.5 mg. per kg. caused complete or nearly complete kill of lice permitted one blood meal on the host. A dosage of 1 mg. per kg. caused a maximum mortality ranging from 18 to 30 per cent. The chemical appeared to be equally effective when administered orally, subcutaneously, intramuscularly, and intravenously. Some kill of engorged lice resulted when the chemical was applied in solution to the unbroken skin of rabbits, but results were erratic. Oral dosage levels of 5 to 100 mg. per kg. produced complete kill of lice for periods ranging from 3 to 8 days. In each case, however, partial kill was obtained for several days after the mortality dropped below 100 per cent. The duration of the toxic principle in the blood was not in relation to the dosage employed, and host variation seemed a greater factor than the dosage level, within the range indicated.

When rabbits were given oral dosages as low as 0.25 mg. per kg. each day for several days, either by forced administration or in food, the blood of the host became toxic to the insects in less than a week, and nearly complete kill of lice permitted one blood meal was obtained. Thus, it was established that the toxic agent was eliminated slowly and accumulated in the blood of rabbits as a result of daily consumption of low dosages of the chemical.

Lice allowed repeated blood meals on rabbits receiving the chemical were killed at dosages even lower than 0.25 mg. per kg. One animal was given 0.1 mg. per kg. in food each day for 19 days. The rabbit food, Purina pellets, contained 5 parts per million of the chemical. Lice which were allowed only one blood meal on the host during this period usually showed less than 20 per cent mortality in 24 hours, but they were killed when allowed blood meals two times each day for three consecutive days. These results proved that the toxic agent accumulated in the insect. The toxic principle persisted in the blood for over 30 days after the animal was taken off the treated food, as evidenced by high mortality rate (usually 80 per cent or more) of lice permitted three day feeding schedules on the animal. During the 44th to the 46th day period after the animal was denied further treated food, the toxic principle was no longer apparent in the blood since over 90 per cent of both male and female lice survived three days of feeding.

Female lice were more readily killed than were the males. It was determined, however, that females consumed in relation to the body weight about two and one-half times as much blood as did the males. This, no doubt, explains the difference in rate of mortality of the sexes.

Lice were affected very rapidly when they engorged on rabbits which received from 25 to 300 mg. per kg. of 2-pivalyl-1,3-indandione. They were dead or paralyzed within 15 minutes to 1 hour after feeding. The toxic agent was shown to be present in the blood of the host as early as 2 hours after administering the chemical and apparently the maximum concentration developed during the first 24 hours even though the toxic principle persisted in the blood for a week or more in most animals.

A limited number of tests indicated that ear mites of rabbits were killed by benzene hexachloride administered to the host at the rate of 100 to 300 mg. per kg. Partial kill of the ear mites resulted from a dosage of 50 mg. per kg. Larvae and nymphs of *A. americanum* (L.) were not affected by this chemical at a dosage level of 25 mg. per kg.

Engorging larvae and nymphs of *A. americanum* were killed on rabbits receiving 2-pivalyl-1,3-indandione at a dosage of 25 mg. per kg. but not at 5 mg. per kg. Some kill of mosquitoes resulted at 25 mg. per kg. but even 300 mg. per kg. did not cause complete kill. This chemical apparently did not affect ear mites when given to the infested host at the rate of 300 mg. per kg.

No special studies were conducted to determine the toxicological effects on rabbits receiving the various chemicals tested, although a number of the materials caused death at the dosage of 300 mg. per kg. 2-Pivalyl-1,3-indandione proved fatal to the host when given at this high dosage and 100 mg. per kg. proved fatal in some cases. No apparent harmful effects were noted when administered orally at the rate of 25 mg. per kg. or less. It is recognized, however, that toxic effects to the host may have been produced at the lower dosage levels even though they were not apparent. Animals on treated food, which provided 0.25 and 0.1 mg. per kg. of the chemical each day for 19 days, showed no harmful effects.

It is believed that the favorable results obtained in the exploratory investigation definitely establish the potentialities of employing insecticides as internal treatments for the control of insect and other arthropod parasites of animals. Further studies against various animal parasites seem warranted, especially with the materials which showed activity as chemotherapeutic agents in connection with these studies.

CAUSE AND POSSIBLE PREVENTION OF THE CRACKING OF THIN CONCRETE WALLS POURED INTEGRALLY WITH ARCH-RIBS AND RIGID FRAMES[1]

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The numerous instances of cracking of thin concrete walls when poured integrally with thick base sections, as exemplified by the spandrel and parapet walls of arches and rigid frame bridges, make expedient the investigation of the cause of these cracks and the setting-forth of means for their control.

When the entire cross-section of the Hartland Bridge is used in making the customary analysis for gravity loads and the usual shrinkage and temperature allowances, it is found that the resulting unit stresses are not the controlling cause of cracking.

Replicas, one-twenty-fourth the size of the Hartland Bridge, show the cause of the cracks to arise within the structure. Even though the stresses produced by the shrinkage of the structure as a unit are eliminated the spandrel walls crack at approximately the quarter-points of the span.

Cracking is believed to be caused by the differential volume change which occurs between the thin wall or stem and the thicker rib or base. Differential volume change is the difference in the free volume changes, i.e., changes in length per unit of length between any two contiguous parts. There are two causes of the differential volume change producing cracking, drying shrinkage and drop in atmospheric temperature. The action of drying shrinkage is shown in the Hartland Bridge replicas.

Tee-sections of plaster-sand mortar and neat plaster are used to illustrate the possibility of producing cracks by differential volume change. One specimen cracked when the drying shrinkage was hastened by oven drying. Another cracked in the stem when a temperature differential was produced by means of refrigeration.

The nature of the stresses for both plain and reinforced concrete tee-sections, as produced by a differential volume change, are determined from the mechanics of differential volume change.

The unit stress in the stem of a plain concrete tee-section, as caused by either drying shrinkage or temperature drop, is

$$s'_1 = \Delta E_c \frac{A_1 A_2}{A_1 + A_2} \left(\frac{1}{A_1} - \frac{hz}{2I} \right)$$

where

Δ = difference in the free volume change of stem and base when considered separated from each other. For shrinkage, Δ = shrinkage of stem

[1] Doctoral thesis No. 813, submitted August 24, 1946.

minus shrinkage of base. For temperature drop, $\Delta =$ (average temperature of stem minus average temperature of base) X coefficient of thermal expansion of concrete.

A_1, A_2 = area of stem and base, respectively.

h = total height of tee-section.

z = distance from centroidal axis to point of stress, positive if above centroidal axis.

I = moment of inertia of tee-section about centroidal axis.

E_c = modulus of elasticity of concrete.

The unit stress in the stem of a reinforced concrete tee-section subjected to drying shrinkage is

$$s'_1 = \frac{p_1 n \Delta'_{F1}}{p_1 n + 1} E_c + \Delta E_c \frac{A'_1 A'_2}{A'_1 + A'_2} \left(\frac{1}{A'_1} - \frac{\bar{a}z}{I_t} \right)$$

where those terms not previously defined are:

$\Delta'_{F1}, \Delta'_{F2}$ = free volume change of stem and base, respectively, if the steel is not bonded to the concrete.

$$\Delta = \frac{\Delta'_{F1}}{p_1 n + 1} - \frac{\Delta'_{F2}}{p'_2 n + 1}$$

p_1, p_2 = steel ratio for stem and base, respectively.

A'_1, A'_2 = transformed area of stem and base, respectively.

$\bar{a}$ = distance between the centroids of A'_1 and A'_2.

$$n = \text{the ratio} \frac{\text{E steel}}{\text{E concrete}} = \frac{E_s}{E_c}.$$

I_t = moment of inertia of transformed section.

The unit stress in the stem of a reinforced tee-section subjected to a drop in atmospheric temperature is

$$s'_1 = -\frac{p_1 n (\lambda_s - \lambda_c)}{p_1 n + 1} E_c + \Delta E_c \frac{A'_1 A'_2}{A'_1 + A'_2} \left(\frac{1}{A'_1} - \frac{\bar{a}z}{I_t} \right)$$

where those terms not previously defined are

λ_s, λ_c = thermal coefficients of expansion of steel and concrete, respectively.

T_1, T_2 = the change in average temperature of stem and base, respectively.

$$\Delta = \frac{(p_1 n \lambda_s + \lambda_c)}{p_1 n + 1} T_1 - \frac{(p_2 n \lambda_s + \lambda_c)}{p_2 n + 1} T_2$$

These stresses are based on a linear stress-strain relationship. In case the use of a non-linear relationship is desired the basic flexure equation is derived and illustrated in the appendices of the thesis.

The analysis of the plain concrete tee-section shows the maximum

unit stress to occur at the juncture of the stem and base and to be appreciably influenced by those factors which affect the value of Δ, notably the width of stem as compared to the thickness of base. The maximum unit stress is approximately $0.75 \Delta E_c$, tension.

The steel ratios, p_1 and p_2, have little influence upon the unit stress in the stem and for all practical purposes the section may be considered to be of plain concrete.

When reasonable values for the differential volume change are assigned to Δ the critical unit stress approaches the ultimate tensile strength of concrete under the combined influence of drying shrinkage and atmospheric temperature drop. In general it is the combined effect of these phenomena and not either one alone which tends to cause cracking.

Curves for the shrinkage of different thicknesses of slabs have been prepared using observed shrinkages and the loss of evaporable water. Values of Δ are obtained from the recommended ultimate shrinkage and the curves showing the differential shrinkage for various slab thicknesses. The field of drying shrinkage has been critically reviewed and evaluated in the appendices.

Average temperature in the different slabs is determined by Schmidt's graphical method and curves showing T_1-T_2 for different temperature drops are shown. The calculated temperature differentials have been verified by field observations on the Hartland Bridge.

The prevention of cracking lies primarily in the control of the amount of Δ resulting from drying shrinkage and in offsetting the effect of shrinkage by construction techniques.

The differential volume change resulting from drying shrinkage may be controlled to a considerable degree by careful choice of cement, mix, and aggregates. The cement should show a low percentage of potential chemical compounds, dicalcium silicate and calcium trialuminate. The mix should be lean and the aggregates non-absorptive and rigid.

The amount of differential volume change resulting from both drying shrinkage and temperature drop may be minimized by making the stem width at least half the base thickness. This step alone will prevent cracking in most instances except where the concrete has disproportionately high shrinkage characteristics.

Certain construction techniques give promise of overcoming the effect of differential volume change caused by either drying shrinkage and/or temperature drop. Among these techniques are the precasting in place, curing and shrinking of the stem and that portion of the base immediately beneath the stem, the employment of pre-stressed steel in the stem, the use of absorptive forms for the stem and the construction of the stem of concrete made from expansive cement.

Stem cracking is the result of differential volume change and cannot be controlled by additional stem steel. The answer lies in the use of selected materials, the selection of stem width at least half the base thickness and possibly in suggested construction techniques.

LINEAR AND CYCLIC AMYLOOLIGOSACCHARIDES AND THEIR RELATIONSHIP TO THE AMYLOLYTIC ENZYMES[1]

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Studies on the straight chain fraction of starch, amylose, are complicated by the refractory nature of the material and the inability to predict its behavior from that of its structural unit, maltose. The desirability of examining homogeneous intermediate molecular weight amylose homologues as a means of simplifying these studies has led a number of investigators to attempt to prepare these compounds. The methods ordinarily used have employed starch as the starting material and involved tedious alcohol fractionations of the acid or enzyme hydrolysate. This investigation presents a method of preparing amyloheptaose, an amylose homologue containing seven glucose units, from Schardinger's β-dextrin. The action of *macerans*, β- and salivary amylases on amyloheptaose and other selected amylooligosaccharides is also reported.

Amyloheptaose was separated from a β-dextrin acid hydrolysate by alcohol precipitation after first removing the β-dextrin. The specific rotation of the material was $[\alpha]_D^{20}$ +175.6°–0.5°. (Theor. 179.6°). The molecular weight determined by the Kline and Acree iodine titration was 1206 (Theor. 1152). The reducing power toward alkaline ferricyanide, analysis of the potassium salt of amyloheptaonic acid and chromatography all indicated that the material was essentially pure heptasaccharide.

Previously proposed mechanisms for the action of β-amylase on amylose chains have been confirmed by the examination of the products of digestion of amyloheptaose, the potassium salt of amyloheptaonic acid, and amylodextrin. β-amylase removes maltose units from the nonreducing end of the molecule converting straight chains with an even number of glucose units entirely to maltose and those with an odd number of glucose residues to maltose and one molecule of amylotriose.

The primary products from the reaction of salivary amylase on amyloheptaose and amylodextrin are maltose and amylotriose. Some glucose is present apparently as a result of the slow hydrolysis of terminal linkages in amylotriose and higher oligosaccharides. Maltose is apparently not hydrolyzed by the salivary amylase preparations used.

The action of *macerans* amylase on amyloheptaose and on Schardinger's α- and β-dextrins alone and in the presence of co-substrates was examined. *Macerans* amylase was found to be capable of attacking amyloheptaose with the production of α-dextrin and glucose, maltose, amylo-

[1] Doctoral thesis No. 840, submitted April 19, 1947.

triose and other oligosaccharides without the production of additional end groups. The enzyme is capable of converting β-dextrin to α-dextrin in the presence of maltose as a co-substrate. Sucrose and gluconic acid do not function as co-substrates for this conversion while the reactivity of glucose is uncertain. β-dextrin alone is stable toward the enzyme. Under similar conditions α-dextrin is converted to β-dextrin with glucose, maltose, calcium maltobionate, α-methylglucoside, sucrose and cellobiose functioning as co-substrates. Some indications were obtained that α-dextrin itself may be slowly attacked. The production of the Schardinger dextrins from linear chains and the interconversion of these dextrins in the presence of co-substrates is accomplished by *macerans* amylase without hydrolysis and the consequent production of new reducing end groups. The enzyme is synthetic in its action as well as degradative, producing linear chains of sufficient length to give detectable iodine colorations. The mechanism of these reactions can be best understood by postulating that the sole function of *macerans* amylase is to promote the exchange of glucosidic linkages between cyclic and linear structures.

THE MORDANTING OF CELLULOSE-ACETATE RAYON, NYLON, AND SILK WITH POTASSIUM DICHROMATE[1]

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The mordanting of three textile fibers, cellulose-acetate rayon, nylon, and silk with aqueous potassium dichromate has been studied as to the effects of initial pH, concentration, and volume of mordanting bath, temperature and duration of mordanting, and initial weight of the fiber to be mordanted. Chromium fixed on each fiber has been estimated from the ash of the mordanted fiber corrected for its initial ash and checked by gravimetric determination of chromium.

Fixation of chromium sesquioxide on cellulose–acetate rayon from fifty volumes of aqueous potassium dichromate during one hour at 100°C. has been shown to be an increasing rectilinear function of initial pH of mordanting bath, 2.0000 g. solute per gram of fiber, between pH 1.17 and 1.29 and a decreasing rectilinear function between this maximum, 4.03 $\pm$ 0.05 per cent, and 0.96 $\pm$ 0.01 per cent Cr_2O_3 at pH 1.67. Loss of acetyl between pH 0.95 and 2.27 was less than 3.8 per cent of its original 38.57 per cent. No change in wet strength occurred when cellulose-acetate rayon was mordanted at the threshold of fixation, pH 2.27; its strength increased upon mordanting at greater pH, approximately 10 per cent at pH 6.47, and decreased by half its first value at pH 0.91.

Fixation on nylon at variable pH and constant concentration, 0.5000 g. solute per gram of fiber, was a decreasing rectilinear function of initial pH of mordanting bath between 10.30 $\pm$ 0.02 per cent Cr_2O_3 at pH 0.93 and 2.54 $\pm$ 0.03 per cent at pH 1.70.

Fixation on silk fibroin from a like bath proved to be an increasing rectilinear function of initial pH from 0.93 to 1.15 and a decreasing rectilinear function between this maximum, 8.59 $\pm$ 0.10 per cent, and 1.30 $\pm$ 0.30 per cent Cr_2O_3 at pH 2.30. The silk lost strength upon mordanting at pH 2.32 or less and retained but 17 per cent of its original wet strength after mordanting at pH 1.21.

Fixation, exhaustion, and the distribution coefficient for each fiber were logarithmic functions of equilibrial concentration, decreasing for nylon but passing through a maximum for cellulose-acetate rayon and silk.

For each fiber, and during 48 hours at 40°C. as well as during one hour at 100°C., fixation of Cr_2O_3 from fifty volumes of aqueous potassium dichromate at constant initial pH was an increasing logarithmic function of equilibrial concentration,

$$x/m = kc^n.$$

[1] Doctoral thesis No. 807, submitted July 20, 1946.

Exhaustion and distribution coefficients at both temperatures were decreasing logarithmic functions of equilibrial concentration. At 40°C. increase in conditioned weight of each fiber upon mordanting, within experimental error of chromic-acid equivalent of fixed Cr_2O_3, and increased absorption of light by mordanted fiber were increasing logarithmic functions of equilibrial concentration.

Fixation of Cr_2O_3 on each fiber during one hour from fifty-volumes of aqueous potassium dichromate (2.0000 g. solute per gram of cellulose-acetate rayon at pH 1.15 or 0.5000 g. solute per gram of silk at pH 1.70) has been shown to be, between 25 and 100°C., an increasing logarithmic function of temperature of mordanting,

$$x/m = kT^n.$$

Exhaustion and distribution coefficient also increased logarithmically with temperature of mordanting.

Fixation of Cr_2O_3 on cellulose-acetate rayon from fifty volumes of potassium dichromate, 2.0000 g. solute per gram of fiber at pH 1.29, was an increasing logarithmic function of time of mordanting,

$$x/m = kt^n$$

between one and 211 hours at 40°C. and between fifteen minutes and four hours at 100°C. At 40°C. the slope of the logarithmic graph of fixation decreased after 49 hours; at 100°C. this change in slope came at 45 minutes. Exhaustion and distribution coefficient were increasing logarithmic functions of time of mordanting at both temperatures and their graphs changed slope like that of fixation. The mean velocity coefficients for the second discontinuous isothermals were less than those for the first. The diffusion coefficients for the first discontinuous isothermal at 40°C. increased to a constant value; at 100°C. the diffusion coefficients passed through a maximum. Until equilibrial pH at 40°C. became constant, in 100 hours at pH 2.00, it increased semilogarithmically as equilibrial concentration of potassium dichromate decreased. No greater loss than 11 per cent occurred during the first hundred hours' mordanting of cellulose-acetate rayon at 40°C.

Fixation of Cr_2O_3 on nylon from fifty volumes of aqueous potassium dichromate, 0.5000 g. solute per gram of fiber at pH 1.15, was a similar increasing logarithmic function of time of mordanting between one and 100 hours at 40°C. and between fifteen minutes and 1.5 hours at 100° C. Exhaustion and distribution coefficient were increasing logarithmic functions of time of mordanting at both temperatures. The velocity coefficient decreased with time at each temperature. At 40°C. the diffusion coefficient remained constant for 64 hours but at 100°C. it rose rapidly. After 49 hours' mordanting at 40°C. and at an equilibrial pH of 1.43 nylon retained but 6 per cent of its original wet strength. Equilibrial pH increased semilogarithmically as equilibrial concentration decreased.

Fixation of Cr_2O_3 on silk fibroin from fifty volumes of aqueous potassium dichromate, 0.5000 g. solute per gram of fiber at pH 1.70, progressed

as an increasing logarithmic function of time of mordanting between one and 229 hours at 40°C. and between fifteen minutes and one hour at 100°C. Exhaustion and the distribution coefficient were increasing logarithmic functions of time of mordanting at each temperature. The velocity coefficient decreased with time. At 40°C. the diffusion coefficient kept constant from four to 169 hours but at 100°C. it continuously increased. Wet strength of silk fibroin upon mordanting 100 hours at 40°C. fell to 39 per cent of its first value while the pH of its mordanting bath rose from 1.70 to 3.40. Computation of energy of activation in calorie/mole from either velocity coefficients or distribution coefficients gave the same values, 13820 for cellulose-acetate rayon, 7655 for nylon, and 9707 for silk.

Fixation of Cr_2O_3 on each fiber,

$$x/m = kv^n$$

and the exhaustion of mordanting bath during one hour at boiling have been shown to be decreasing logarithmic functions of initial volume of mordanting bath, 2.0000 g. solute per gram of cellulose-acetate rayon or 0.5000 g. solute per gram of nylon or silk, with initial pH of the shortest bath rising upon dilution. The distribution coefficient, until it became constant, was also a logarithmic function of initial volume, decreasing for cellulose-acetate rayon and nylon but increasing for silk. When cellulose-acetate rayon and nylon were mordanted in another set of mordanting baths, like the first except for constant initial pH, fixation and exhaustion decreased, but not logarithmically, with volume of mordanting bath and distribution coefficient increased to a constant value.

Fixation of Cr_2O_3 on each fiber during one hour at 100°C. from a constant volume of aqueous potassium dichromate (2.0000 g. solute per gram of cellulose-acetate rayon, 1.5000 g. solute per gram of nylon, or 0.5000 g. solute per gram of silk fibroin) was a decreasing logarithmic function of initial weight of fiber between one and thirteen grams,

$$x/m = km^n.$$

Exhaustion was an increasing logarithmic function of weight of fiber. For nylon the distribution coefficient was an increasing, but for cellulose-acetate rayon and silk a decreasing, logarithmic function of weight of fiber.

AMYLOSE COMPLEXES WITH ORGANIC ACIDS AND ALKYL HALIDES[1]

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Appreciable amounts of fatty acids are known to be associated with cereal starches. Although several hypotheses as to the nature of the interaction of the fatty acids with starch and the nature of the forces binding these acids to starch have been proposed, not any seemed entirely satisfactory.

In this work it is shown that fatty acids, dicarboxylic acids and alkyl halides form complexes with amylose, the unbranched component of starch. X-ray diffraction patterns produced by these complexes indicate a crystalline structure similar to that of the complexes which amylose forms with iodine and the alcohols (1, 2). The structure consists of close packed helical amylose chains with the molecules of the complexing agents within the helices. The packing of the amylose is not materially influenced by the complexing agents, indicating that the complexing agents are contained in spaces within the structure of the amylose. Of the two possible positions, the space within the helices and the interstitial space, the former appears to be more likely.

Neither the chain length nor unsaturation of the fatty acid has any effect on the structure of the complex. However, a difference in the X-ray diffraction patterns of the wet and partially dried amylose-fatty acid complexes and of the thoroughly dried complex is noted. The X-ray lines of the latter are definitely shifted to larger angles. In the wet and partially dried samples, the amylose helix has a diameter of 13.7 Å. In the thoroughly dried complex, the helix has a diameter of 13.0 Å, equal to that found for the helix in the amylose-iodine complex (1).

Results are given of a study of the effect of varying amounts of lauric, palmitic, oleic and stearic acids on the iodine binding power of amylose as determined by the potentiometric iodine titration (3). Inhibition of amylose-iodine complex formation is proportional to the fatty acid content of the amylose. This is attributed to a decrease in the capacity of the helices for iodine when the helices contain fatty acids. Evidence is presented that there is a high degree of correlation between the amount of fatty acid bound by helical amylose and the calculated capacity of the interior of the amylose helix. The experimental binding power is from 88 per cent to 91 per cent of the fatty acid calculated on the basis of the helical structure.

Fatty acids can be displaced from the amylose-fatty acid complex by iodine vapors at 70°C. The amounts of fatty acid displaced and the iodine

[1] Doctoral thesis No. 808, submitted July 20, 1946.

gained by the amylose are proportional in the spatial or steric sense. The reversibility of the reaction,

$$\text{Amylose}\cdot I_2\,(s) + \text{Fatty Acid} \rightleftharpoons \text{Amylose}\cdot\text{Fatty Acid}\,(s) + I_2$$

is clearly shown. In aqueous solutions, the equilibrium lies far to the right whereas with dry solids and iodine in the vapor phase, where the iodine activity is greater, the equilibrium is shifted in the opposite direction.

Amylose in the helical "V" configuration binds large amounts of fatty acids when treated with methanol-fatty acid solutions. Retrograded "B" amylose and amylopectin, having essentially the same surface as helical amylose, do not bind significant amounts. This indicates that crystalline configuration is a more important factor than surface in determining whether amylose will bind fatty acids. Adsorption cannot be used satisfactorily to explain the variations mentioned above.

The small amount of fatty acids taken up by amylopectin and the small amount of fatty acids found in the granules of waxy corn starch (which is essentially amylopectin), can be explained very well by the helical hypothesis. Amylopectin, being highly branched, is not capable of forming helices; hence it is not capable of binding the fatty acids.

Amylose in the helical "V" configuration binds an appreciable amount of palmitic acid from a carbon tetrachloride solution low in palmitic acid concentration. Relatively large concentrations of fatty acids are necessary for the introduction of fatty acids into helical amylose from methanol solutions. Methanol is capable of entering the helices of amylose and competing with the fatty acids for space, whereas carbon tetrachloride is not. This also explains the ability of methanol to extract fatty acids from amylose and the inability of carbon tetrachloride to do so. In addition to being able to dissolve fatty acids, a solvent must be capable of displacing the fatty acids from the complexes.

Soaps and other complexing agents will completely precipitate partially dispersed starch. The factors which determine the completeness of precipitation are (1) solubility of the soap and (2) degree of dispersion of the starch granules. Evidence is presented which indicates that in granular starch the amylose and amylopectin are mixed intimately throughout the granules.

Dicarboxylic acids, ethyl adipate and hexamethylene glycol, molecules which have no electric moment parallel to the long axis, form amylose complexes similar to those of iodine, butanol and fatty acids. Hence it is concluded that the dipolar molecules do not seem to be necessary for complex formation with amylose.

Thermal stability of the dicarboxylic acid amylose complexes depend on the chain lengths of the acids. The longer the chain the greater the thermal stability. Partial separation of adipic acid from sebacic acid in mixtures of the two was effected by treating with amylose at 50°C. The greater portion of the sebacic acid precipitated as the amylose complex while most of the adipic acid remained in solution.

Hydrogen bond formation cannot be used to explain the difference

in complexing ability of amylose and amylopectin. Each has essentially the same groups available for bond formation, yet the one forms complexes while the other does not. Neither can hydrogen bond formation be used to explain the interaction between amylose and alkyl halides.

The X-ray diffraction patterns produced by amylose complexes of normal hydrocarbon alkyl halides are identical with those produced by the amylose-butanol and amylose-iodine complexes. The patterns produced by branched alkyl halides are identical with those produced by branched alcohols (4).

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THE NITROGEN FACTOR IN SWEET POTATO PRODUCTION IN IOWA[1]

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Sweet potato production has been an important enterprise on the sandy soils of southeastern Iowa since 1865. The largest production area of 1,500 acres is located in the Fruitland district of Muscatine County, with smaller acreages located near Conesville, Burlington, Montrose and Carlisle. A sound fertilization program has been an important problem of these growers for many years, and although previous investigations had shown the value of phosphorus and potash, little had been done concerning the role of nitrogen.

The literature relative to the problem indicates that nitrogen applications have increased yields on most sandy soils devoted to sweet potato culture and on some of the heavier textured soils of the south where leaching is an important factor. Shape and the chemical composition of the roots are reported as not being altered by nitrogen applications.

In this study nitrogen fertilizers were tested for their effect on the yield, grade, storage quality and chemical composition of sweet potatoes. The effect of nitrogen on production was studied by means of field tests established on the Muscatine Island Field Station located near Fruitland, with the following general experiments being conducted:

1. A factorial experiment with three levels each of nitrogen, phosphorus and potassium, designed for the purpose of establishing the proper rate and balance of nitrogen when in combination with other nutrients.
2. A time of nitrogen application experiment designed to test the result of early, late and split applications of nitrogen on yield and quality.
3. A nitrogen carrier test outlined to ascertain the most suitable source of nitrogen for the conditions involved.
4. Miscellaneous experiments to compare manure and commercial fertilizers and to test the value of nitrogen in starter solutions.

Sweet potatoes from the various treatments in Experiment 2 were placed in storage in 1938 and 1939 for the purpose of determining the effect of nitrogen on the keeping quality of the roots when applied at different intervals during the growing season. Potatoes from the same treatments were collected and analyzed for sugar, starch and nitrogen content.

At the beginning of the investigation a preliminary survey was made on the nitrate, total nitrogen and organic matter content, and the moisture constants of the soil.

[1] Doctoral thesis No. 641, submitted July 12, 1941.

The field experiments were laid out in approved designs and the data were subjected to appropriate statistical analysis. Fertilizers applied before planting were placed in bands 8 to 10 inches deep in the center of the ridge, while those applied as sidedressings were broadcast by hand along the side of the ridge and cultivated in. Observations were kept on relative foliage color and rate of vine growth; yield records were obtained by recording the weights of No. 1, No. 2 and cull grades and any differences in shape, color, or degree of maturity of the roots were noted.

Rates of applications in Experiment 1 ranged from 22.5 to 75 pounds of nitrogen per acre, 20 to 52.8 pounds of phosphorus and 54 to 108 pounds of potassium. Since nitrogen was the variable in the other experiments, all of the plots received approximately 90 pounds each of P_2O_5 and K_2O before planting. Except for the check treatments which received none, nitrogen was supplied at the rate of 45 pounds per acre in Experiments 2 and 3. Aside from the check and a treatment receiving all of the nitrogen before planting, the treatments in Experiment 2, where applications were made at monthly intervals, were extended from the time of planting to two months after planting. The nitrogen carriers tested in Experiment 3 were nitrate of soda, ammonium sulfate, Uramon, calcium cyanamid and calcium nitrate.

For the storage tests one bushel, 50 pounds, of No. 1 grade roots was selected from each replication of each treatment of Experiment 2 and stored in bushel baskets in a special room in a local commercial storage house. The following March, the lots were examined for shrinkage and losses due to rots.

For the physiological tests, 30 potatoes were selected at random from each set of treatments in Experiment 2 and, from these, 100 gram samples were killed and stored in 80 per cent alcohol until the analyses for sugars, starch and nitrogen were run. Samples for the 1938 crop were taken from the storage lots in March; but for the 1939 crop, the samples were collected in the fall, cured for 3 weeks, then killed in alcohol.

Both the 1938 and the 1939 seasons had periods of comparatively heavy rainfall and the total for the season was well above average, whereas 1940 was relatively dry throughout, except for a wet week about the middle of August.

The soil in the Fruitland district is classed as a Buckner coarse sand, which is very porous and is underlain with gravel. This soil is very low in total nitrogen, organic matter, base exchange capacity and water holding capacity. The soil is fairly acid, ranging from pH 4.6 to 5.5.

During the three years of this investigation, results in the field tests were obtained as follows:

Experiment 1. Nitrogen applications in two of the three seasons improved the color and growth of the vines, increased yields and produced a higher percentage of U. S. No. 1 grade potatoes. In the dry season, 1940, although vine growth was improved, nitrogen fertilizers either failed to show any beneficial effect on or caused a depression of yields. Amounts between 25 and 45 pounds of nitrogen per acre were adequate in those

years of heavy rainfall, when the applications proved beneficial. Phosphorus and potassium applications proved highly essential in production of sweet potatoes on these soils. The most economical rates were approximately 20 pounds of phosphorus and 75 pounds of potassium per acre.

Experiment 2. The results of these tests indicate that sweet potatoes are rather sensitive to the supply of nitrates in the soil. Yield responses from nitrogen fertilizers were obtained whenever leaching of the natural soil nitrates occurred from spring or early summer rains. Since applications in years of abnormally low rainfall were detrimental in some cases, applications of nitrogen should be made after the character of the season has begun to unfold. In the event of fairly heavy spring rains, an initial application of 25 pounds of nitrogen per acre should be made three to four weeks after planting. With abnormally heavy summer precipitation, a second application midway in the growing season should prove beneficial.

Experiment 3. Nitrate of soda and ammonium sulfate were satisfactory for either preplanting or sidedress application, but as may be expected, the nitrogen in these inorganic forms was subject to leaching. Although calcium cyanamid was very satisfactory in the wet years when applied two weeks prior to planting, it was distinctly injurious in 1940, the dry year. Also the toxic properties of this material make it unsuited for sidedress applications even in years of heavy precipitation.

Experiment 4. Barnyard manures in general were not as satisfactory as commercial fertilizers. Starter solutions used at the time of transplanting gave the plants a faster start, but the effect was of short duration and did not increase the yield.

The results of storage tests showed that applications of nitrogen did not impair the keeping qualities of sweet potatoes, and in the 1939 trial there were fewer rots in the lots from treatments receiving late applications than from the check or the treatment receiving all of the nitrogen before planting.

The sugar and starch content of the sweet potatoes was not altered by the nitrogen treatments, regardless of the time of application. Nitrogen applications materially increased the colloidal nitrogen content of the roots, particularly when applied a month or two after planting.

In summary, nitrogen is an important factor in the production of sweet potatoes in the Fruitland district. Since sweet potatoes are sensitive to an oversupply of nitrates in the soil, applications must be made judiciously, usually at the rate of 25 pounds of nitrogen per acre, applied in an inorganic form and as sidedressings after periods of rather heavy rainfall.

THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED MILK[1]

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Interest in the manufacture of blue cheese from pasteurized milk is increasing rapidly. Pasteurization is desirable for the control of certain microbial defects, such as gasiness and unclean flavors, as well as from the public health standpoint.

A study was made of some possible means by which blue cheese could be made from pasteurized milk and still develop the flavor characteristic of the product made from raw milk. Previous studies have indicated the effect of pasteurization is attributable, to a large degree, to the inactivation of the normal milk lipase, resulting in a decrease in fat degradation in the cheese made from pasteurized milk. Attention therefore was centered upon means for obtaining an increased production of free fatty acids and the ketones derived therefrom in blue cheese made from pasteurized milk.

A total of 143 lots of cheese was made. Seven lots were made with the addition of varying amounts of the lower molecular weight fatty acids. The addition of the acids improved the flavor of the cheese but did not give the flavor typical of good blue cheese.

The addition of cultures of three lipolytic bacteria and one lipolytic yeast to milk for cheese making was tried. Cultures of the organisms grown in cream containing 18 per cent fat were tried in 16 lots of cheese; the same organisms grown in homogenized whole milk were used in 16 lots of cheese. In both series *Mycotorula lipolytica* improved the flavor of the cheese and in some cases caused excessive fat breakdown. *Alcaligenes lipolyticus* gave the cheese unnatural flavors and did not cause appreciable fat degradation. The cheese made with *Achromobacter lipolyticum* and *Pseudomonas fragi* added to the milk were musty and unclean, and showed no evidence of fat hydrolysis by the organisms.

Eleven strains of *Mycotorula lipolytica* isolated from various dairy products at different times were used in 28 lots of cheese to determine the effects of different strains of the organism. Most strains improved the flavor of the cheese, but strains 839, 843, M.L., 848, and 846 gave the most improvement in flavor. These same strains in slightly different order gave the most improvement in flavor in second trials.

A collection of 18 strains of mold of the *Penicillium roqueforti* type was assembled from various sources. These were tested for lipolytic ability by the nile-blue sulfate technique and for proteolytic ability by Freudenreich's technique on Czapek's agar. Wide variations in lipolytic

[1] Doctoral thesis No. 849, submitted June 7, 1947.

and proteolytic ability were shown by the different cultures on regular Czapek's agar. The lipolytic ability of the cultures was much greater and about equal for all cultures when the sugar in Czapek's agar was replaced by peptone.

From the results of the tests reported in the preceding paragraph, eight strains were chosen, and a lot of cheese from homogenized raw milk and one from homogenized pasteurized milk were made with each strain. No strain was found to be especially suited to the manufacture of blue cheese from pasteurized milk. Strains 6 and 7 gave the best results in both types of cheese, and strain 17 gave the best result in cheese made from homogenized raw milk.

The addition of an emulsion of a well-cured, fine-flavored cheese to the milk from which blue cheese was made proved very unsuccessful, apparently due to the transfer of contaminating microorganisms, presumably yeasts, to the milk and subsequent development of a yeasty flavor defect in the cheese.

A mold-enzyme preparation was made by the growth of *Penicillium roqueforti* on a modified Czapek's medium which was semi-solid, low in carbohydrate content, and contained 10 per cent butterfat. This was added to the milk at the rate of 0.55 per cent and gave cheese with a very high flavor and excessive rancidity after curing for 12 weeks. Similar preparations were made with five strains of mold of the *Penicillium roqueforti* type and added to milk in amounts varying from 0.05 to 0.25 per cent. All strains brought about an improvement in flavor, although some improved the flavor more than others. The optimum amount of mold-enzyme preparation to use was between 0.10 and 0.25 per cent, depending upon the sharpness of flavor desired. Strains 4, 12, and 6 gave the greatest improvement in flavor, while strain 13 gave the least improvement in flavor.

Protein degradation studies were made on a portion of the trials in an attempt to correlate protein breakdown with flavor development. Little or no correlation of values for amino nitrogen, nitrogen fraction soluble in trichloroacetic acid, and nitrogen fraction soluble in phosphotungstic acid with the flavor scores of the cheese was found.

Fat acidity and total volatile acidity were determined in an attempt to correlate fat degradation with flavor development. Fat acidity showed no apparent relation to the flavor score of blue cheese. The values for total volatile acidity correlated fairly well with the flavor scores. The total volatile acidity was the only chemical determination employed which could be used to predict the flavor score with any degree of precision. Cheese with total volatile acidities in the range in which 30 to 55 ml. of 0.1 *N*. alkali were required to titrate the volatile acids in the first 1000 ml. of steam distillate from 200 g. of cheese, generally had the highest flavor scores.

THE LIPOLYTIC ENZYME SYSTEM OF *MYCOTORULA LIPOLYTICA*[1]

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Pasteurization of milk is known to destroy milk lipase, which is essential in the normal ripening of blue cheese. This study was undertaken to explore the possibilities of substituting a microbial lipase for the normal milk lipase in the manufacture of blue cheese from pasteurized milk.

Lipase activity was determined by allowing the enzyme to react upon 5 per cent butterfat, emulsified in semi-solid agar, for 48 hours at 37° C., after which the liberated free fatty acids were titrated with N/20 potassium hydroxide in methyl alcohol with phenolphthalein as indicator.

Mycotorula lipolytica was selected for a study of its lipolytic enzyme system because this microorganism possessed strong lipolytic and weak oxidative properties and produced considerably more lipase in nutrient broth than either *Achromobacter lipolyticum* or *Pseudomonas fragi.*

Of the five growth media studied in the early phases of the investigation, nutrient broth containing 0.05 per cent glucose was most suitable for lipase production by *M. lipolytica.* Partially-defined synthetic media and skim milk or whole homogenized milk proved much less satisfactory.

Lipase production by *M. lipolytica* took place at pH levels from pH 3.5 to 8.5 with pH 4.5–5.5 as optimum. The addition of 0.225 m disodium phosphate plus citric acid was sufficient to maintain a constant pH level over growth periods of from 4 to 6 days. An equal addition of disodium phosphate plus monopotassium phosphate gave similar results. A sodium acetate plus acetic acid buffer was unsatisfactory. The presence of 0.450 and 0.675 m disodium phosphate plus citric acid was even more favorable for lipase accumulation than was a concentration of 0.225 m at the same reaction of the medium.

Growth of the organism and lipase production took place at temperatures from 21° to 36° C,, with 30° C. as optimum for the production of lipase, while highest counts were obtained at 25° C.

The accumulation of lipase in the medium was favored by growing the culture without shaking. Continuous aeration of growing cultures resulted in but little lipase production, while the growth rate of the cells was considerably increased as compared with non-aerated controls.

In general short oval cells and low lipase activity were associated with conditions favorable for rapid growth, while long oval and slender cells and high lipase activity were associated with conditions slightly less favorable for growth.

[1] Doctoral thesis No. 855, submitted June 9, 1947.

Lipase activity on butterfat was demonstrated over a range of reaction from pH 4.0 to pH 8.0, with pH 6.2–6.5 as optimum.

Activity was demonstrated at temperatures from 10° to 52° C. Temperatures of 37° C. and above tended to inactivate the enzyme, the effect being more pronounced with higher temperatures. At 10° C. lipase activity continued at a relatively low but constant rate over a 30-day period.

A storage temperature of 5° C. was much superior to 25° C. for preserving the enzyme in cell-free preparations, activity being maintained at a high level for at least 3 months when storage was at the lower temperature in closed containers.

Lyophilized enzyme preparations retained about half their original total lipase activity and had a high enzyme concentration per unit weight. Considerable concentration of lipase by foaming with carbon dioxide was demonstrated; high losses of enzyme occurred during the process.

Salting-out of lipase with saturated ammonium sulfate plus 30 per cent of 95 per cent ethyl alcohol resulted in a white precipitate high in lipase activity. The activity of this preparation represented only a very small portion of the original lipase activity of the culture. Treatments at 6° C. resulted in more active preparations than treatments at 24° C.

The beneficial effect of cell-free preparations of lipolytic enzyme from *M. lipolytica* upon the ripening of blue cheese made from pasteurized homogenized milk was observed by organoleptic tests as well as by determinations of steam-distillable volatile acid on cheese ripened for 4 and 12 weeks. This enzyme was considered of potential value in the production of blue cheese from pasteurized milk on a commercial scale.

EVALUATION OF AMYLOLYTIC AGENTS EMPLOYED IN THE ALCOHOLIC FERMENTATION[1]

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Chemical tests previously devised for determining the enzymic activity of amylolytic agents include polarimetric, iodometric, reducing power, and viscosity measurements. In general these chemical tests measure the α-amylase, β-amylase, or the total α- and β-amylase activity of the agent employed, and, although they afford rapid measures of the potencies, there can be found no correlation between any of these test methods and actual performance in saccharifying fermentation mashes, particularly with the fungal amylase products known as mold brans. This investigation was initiated because of the lack of correlation of the chemical tests and the need for a test for fungal amylase products prepared with strains of *Aspergillus oryzae* that would have all the advantages and merits of the most conclusive evaluation method, namely, the tedious three to four day conventional fermentation, but would require a shorter period of time and give an exact fermentative evaluation.

Graphical and mathematical analysis of meager fermentation data covering only limited levels of mold bran and ethanol yields from the conventional 72-hour corn mash fermentations indicated a straight line function. This straight line function was obtained by plotting $\frac{\text{weight of mold bran}}{\text{weight of ethanol}}$ against the weight of mold bran. Based upon this graphical and mathematical principle a rapid and simple 24-hour fermentation test procedure was developed, which was designated as the "Standard Evaluation Test."

After extended investigations of corn mashes and food grade corn starch media of three starch concentrations, the test medium adopted as most satisfactory for the Standard Evaluation Test contained 100.0 g. of corn starch and 5.0 g. of Difco yeast extract in each wide-mouthed 1-liter Erlenmeyer flask. These ingredients were mixed with 250 ml. of 0.05 normal hydrochloric acid, the starch gelatinized by heating in a water bath with continuous stirring, and the mashes cooked for 60 minutes at 20 pounds steam pressure. To the hot mash in each flask were added concentrated sodium carbonate solution to adjust the pH between 5.0 and 5.3 and the amylolytic agent under test as a slurry in 250 ml. of cold water of such temperature that the resultant mash temperature, after mixing for one minute with a high speed mixer, was about 55° C. Four

[1] Doctoral thesis No. 852, submitted June 9, 1947.

levels of 1.0 g., 2.0 g., 3.0 g., and 4.0 g. of mold bran were used in triplicate in each series. The mashes were cooled to 30° C., inoculated with 20 ml. of a 24-hour yeast culture and incubated at 30° C. for 24 hours, after which the alcohol content for each flask was determined by distillation and subsequent specific gravity measurement. The data were plotted as $\frac{\text{weight of mold bran}}{\text{weight of ethanol}}$ against weight of mold bran. When the curves were extrapolated to the Y-axis parallel straight lines having different numerical intercepts were obtained, the intercepts being inversely proportional to the relative amylolytic activity of the various agents employed. This relationship held for the 17 samples, of widely differing amylolytic activity, including laboratory, pilot plant, semi-commercial, plant experimental, and commercial samples, which were tested and evaluated. The intercepts obtained from the curves are shown in Table 1.

SUMMARIZED DATA OBTAINED WITH THE STANDARD EVALUATION TEST PROCEDURE

Mold Bran	Type	Graphical Intercept Value	Minimum Optimal Levels for Maximum Ethanol Production	
			Calculated	Experimental
FCPC No. 1...	Laboratory	0.0100	2.00	2.5
MB No. 9.....	Commercial	0.0131	2.62	2.6
SLI.....	Commercial	0.0132	2.64	2.6
FCPC No. 2...	Semi-commer.	0.0133	(2.66)*	2.66
JWC.....	Commercial	0.0144	2.88	3.0
UN.....	Pilot plant	0.0156	3.12	3.5
MB No. 2.....	Concentrate	0.0163	3.26	.....
MB No. 4.....	Plant exper.	0.0177	3.54	.....
MB No. 1.....	Commercial	0.0200	4.00	4.0
MB No. 10....	Commercial	0.0210	4.20	4.2
MB No. 11....	Commercial	0.0213	4.26	4.3
MB No. 5.....	Plant exper.	0.0236	4.72	.....
ISC No. 2.....	Laboratory	0.0240	4.80	4.8
MB No. 6.....	Plant exper.	0.0250	5.00	.....
ISC No. 1.....	Laboratory	0.0264	5.28	5.3
MB No. 3.....	Plant exper.	0.0303	6.06	.....
MB No. 12....	Commercial	0.0378	7.56	7.6

* Determined experimentally.

Since the slopes from all the test curves obtained in the Standard Evaluation Tests had practically the same value of about 0.0274, an alternate procedure was found possible employing a single level of 3.0 g. of mold bran per flask instead of four levels. The intercept was then calculated from the straight line equation, $y = mx + b$. Although probably not quite as accurate as the Standard Evaluation Test using four levels of amylolytic agent, this one-level method was sufficiently accurate for commercial laboratory control work.

Using the Standard Evaluation Test procedure an exact fermentation evaluation was thus possible, since as the values of the intercepts vary between two agents, the minimum optimal levels for maximum ethanol production vary in the same ratio. Correlation of the intercept values from the Standard Evaluation Tests with the minimum optimal requirements for maximum ethanol production was made by first carrying out conventional 72-hour corn fermentations with various levels of a standard reference sample to ascertain the minimum optimal level of this sample for maximum ethanol yields. Then the proportion

$$\frac{\text{intercept (standard agent)}}{\text{intercept (unknown agent)}} = \frac{\text{optimum level (standard agent)}}{\text{optimum level (unknown agent)}}$$

was employed to compute the minimum optimal level for maximum ethanol production for the other amylolytic agents evaluated. The calculated values are shown in Table 1. Finally to confirm the computed minimum optimal level of each agent actual maximum ethanol production data were obtained for most samples from conventional 72-hour corn fermentations employing six different levels of each amylolytic agent, over a range which included the computed minimum optimal level. The optimum levels thus determined experimentally are also shown in Table 1. The computed and experimentally determined minimum optimal levels for most of the samples gave excellent agreement.

The rapid evaluation of fungal amylolytic agents as to their relative activities and also as to their minimum optimal requirements for maximum ethanol production is thus possible. Any good agent is selected as a reference standard, and the intercept and numerical value for the slope obtained for this reference material by the Standard Evaluation Test. Conventional 72-hour grain fermentations are then carried out employing various levels of the same reference agent to determine the minimum optimal requirement for maximum ethanol production. To evaluate any other agent the intercept is obtained, either graphically by plotting data from the regular Standard Evaluation Test employing four levels of agent, or calculated from the straight line equation employing single-level data. From the intercept the minimum optimal requirement is then computed from the proportion:

$$\frac{\text{intercept (standard agent)}}{\text{intercept (unknown agent)}} = \frac{\text{optimum level (standard agent)}}{\text{optimum level (unknown agent)}}.$$

THE LIFE HISTORY AND MANAGEMENT OF THE WHITE BASS, *LEPIBEMA CHRYSOPS* (RAFINESQUE), IN SPIRIT LAKE, IOWA[1]

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While the natural range of the white bass (*Lepibema chrysops*) presumably does not extend west of the Mississippi River drainage, the largest concentration of this fish in Iowa is in the Okoboji Lake chain, a branch of the Missouri River system. According to records in the Iowa State Conservation Commission files, 100,000 white bass were stocked in the Okoboji Lake chain in 1898. Whether or not there were white bass in Spirit Lake before this stocking is not known, but it appears improbable. White bass have been taken from the lake as long as some of the older residents can remember, and the population has ranged from one of the most abundant fish in the lake to near extinction. Since 1939 it has appeared prominently in the catch, and has presumably been of considerable influence in the ecology of the lake.

The study of the Spirit Lake white bass was started in 1942, and the field work was completed in 1946. In addition to the material collected by the writer, information on 22 white bass was used from the 1941 collections of Max Davis[2] and Tom Moen. Dr. R. M. Bailey collected 31 specimens for the project between April and July, 1943. No collections were made in 1944. The study of the Spirit Lake white bass was based on fish taken in 1941, 1942, 1943, 1945, and 1946. The data were obtained from the following number of specimens: Body-scale ratio, 1,142; length-weight relationship, 1,459; analyses of stomach contents, 972.

The collection of the young-of-the-year Spirit Lake white bass was made largely with a 400-foot seine equipped with a ½ inch stretched-measure bag and with ¾ inch stretched-measure wings. The white bass, other than the young fish, were taken by hook and line, by means of gill-nets, and with seines ranging in length from 25 feet to 2,000 feet. The two types of gill-nets used during the investigation were commercial nets similar to those commonly used by the local fishermen, and experimental nets.

Spirit Lake is a shallow, rich, hard water, eutrophic lake not exceeding 23 feet in depth, except during short intervals. Spirit Lake and its watershed are covered by Wisconsin glacial drift. The normal temperature for the Northwest Iowa District is 46.6 degrees Fahrenheit, the normal precipitation is 28.40 inches, and the normal growing season is 148 days. *Potamogeton richardsonii* and *Scirpus acutus* are the dominant

[1] Doctoral thesis No. 846, submitted June 6, 1947.
[2] Killed in action, 1944.

deep-water plants. The fluctuations in the water level elevations in the last 13 years apparently have not exceeded nine feet. A combination of silting and decomposition of organic material has leveled the lake bed and decreased the average depth in the last 30 years. Siltation of the lake, as a result of agricultural activities, probably is a minor factor.

An estimate of the relative abundance of the more common Spirit Lake fish was made by evaluative information from rough-fish removal operations, gill-netting catches, test-seining results, hook and line fishing, and a dead fish survey. In general, the young of the pan and game fish amounted to a very large percentage of all of the small fish in the lake. The young of yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigro-maculatus*), common bluegill (*Lepomis macrochirus macrochirus*), and northern black bullhead (*Ameiurus melas melas*) accounted for almost all of the small pan fish. Young-of-the-year white bass were quite common in 1942; young largemouth bass (*Huro salmoides*) were somewhat less common in 1945 and 1946. The number of small pan fish was always many times greater than that of the young game fish. Adults of yellow perch and black bullhead were very abundant for every year considered. The carp (*Cyprinus carpio*) was the most common non-game food fish. The two species of gar, the shortnose (*Lepisosteus platostomus*) and the northern longnose (*Lepisosteus osseus oxyurus*), were not common. Etheostominae were more numerous than native Cyprinidae, but neither were common. The walleye (*Stizostedion vitreum vitreum*), and the northern pike (*Esox lucius*), were the most common of the larger game fish.

The average spawning date for white bass in 1946 was believed to have been sometime during the last week in May. The water temperature for this period was approximately 60 degrees Fahrenheit. Attempts to obtain and fertilize eggs for incubation in the fish hatchery met with uniform failure. Mature fish in two small ponds apparently did not reproduce successfully.

The body-scale relationship can be described as a regression line having a Y intercept (length axis) of 23.89 and a slope of 1.883. The females grew faster and larger than the males, but they did not live as long. The 1941 year-class dominated the population from 1942 through 1946. The average growing season is estimated to be between 125 and 150 days. The most rapid growths occur in June, July, and August. The average date of annulus completion for the 1941 year-class in 1945 was early in August. There was virtually no growth of the 1941 year-class in 1946. While none of the fish was mature at the end of their first year, apparently all, or almost all, were adult at the end of three years. A larger number of females than males were mature at the end of the second year of life. The mathematical relationship between the length and weight, for all data combined, may be expressed by the equation:

$$W = 9.688 \times 10^{-4} L.^{3.206}$$

The coefficient of condition increased irregularly as the length increased regardless of sex or age.

The Spirit Lake white bass fed almost entirely on fish, insects, and crustaceans. The bluegill and the black crappie composed the bulk of the fish food. Game fish were an unimportant item. Non-game food fish, excluding the freshwater sheepshead (*Aplodinotus grunniens*), were not taken. The two orders of insects taken most often were Diptera and Ephemeroptera. *Leptodora kindti* was the most frequently determined crustacean. The larger white bass tended to feed more on fish, while the young fed largely on invertebrates. There was little or no feeding activity under the ice. With the advent of warm weather the white bass remained in deep water during the day, and moved inshore at twilight.

Abundant year-classes of white bass should be harvested rapidly with little or no restriction on either the size or the number taken. During periods of low populations the season should remain closed on immature fish and adult fish that have not spawned during the current year. Fish just reaching maturity rather than adults nearing senility should be used for stocking purposes. Careful consideration should be given to the possible effects on more desirable game fish before white bass are introduced into a lake or stream.

SOME FACTORS AFFECTING THE FUNCTIONAL PROPERTIES OF LIQUID EGG ALBUMEN[1]

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A study has been made of some factors affecting the beating and leavening properties of egg albumen.

A whip test, which fairly accurately measures the property of egg albumen to perform in angel cake was devised and used. This test was an accurate measure of changes in egg white resulting from certain treatments such as heating, acidification, fermentation and homogenization. The test was capable of showing slight differences in treatment and correlated fairly closely with actual performance of the white in angel cake. However, many factors affect the relationship between the whipping test and the angel cake performance. Good correlation holds only under certain specified and tested conditions to certain limitations. It is necessary to determine the correlation of the whip test and angel cake performance with each specific treatment so that erroneous conclusions will not be drawn.

It was found that fresh egg white loses its foaming property when heated to 142° F. Holding periods in excess of 30 seconds at 140° F. resulted in a loss in angel cake performance. Holding times at a temperature of 120° F. for one hour or more resulted in similar losses in beating and cake performance.

Lowering the pH of egg white from 8.5 to 7.0–6.0 by acid results in a slight loss of beating capacity. However, the stability to heat is definitely increased by such adjustments. Fermentation also lowers the pH of egg white but the loss of beating power in this case is not observed until a pH of 6.6 or lower is reached.

The addition of sugars to egg white resulted in a definite beneficial effect on its functional properties when subjected to various treatments. Sucrose exhibited a marked heat stabilizing effect on egg white. The stabilization to heat increased with increase in sugar concentration.

It was also shown that at the same concentrations, glucose, lactose and maltose were more effective in stabilizing the egg white to heat than were sucrose and mannitol. Glycerol exhibited the same effect as did sucrose. Ethylene glycol, triethylene glycol and polyethylene glycol, although increasing the beating power of egg white prior to heat treatment had an adverse effect on heating. The loss of whipping property was greater on heating when these glycols were added than when the egg white was heated alone.

The addition of sugars to egg white increased the whipping power.

[1] Doctoral thesis No. 823, submitted December 13, 1946.

Sugar also permitted the pH of egg white to be lowered to 6.5 without impairing the initial foaming capacity.

The addition of sodium chloride in concentrations up to 1.0 per cent had no effect on the initial foaming rate of egg white. When subjected to heat, however, these low concentrations did show a slight stabilizing effect.

It is suggested that the results of this study indicate that mucin plays an important role in the whipping property and angel cake performance of egg white. Adjusting the pH to near the isoelectric point of mucin results in a loss in cake performance. In addition when thick white (containing a majority of mucin) is heated a greater loss in whipping property occurred than when heating thin white.

Homogenization resulted in a definite loss of whipping capacity; this effect varied with the homogenization pressure used. The addition of sugar and lowering the pH did not have any beneficial effect when homogenization was used. Angel cake performance did not always correlate with the beating test. For instance, satisfactory cakes were made from egg whites homogenized at low pressures. However, when high homogenizing pressures were used unsatisfactory cake performance resulted.

THE SYNTHESIS, RESOLUTION, AND METABOLISM OF TYROSINE ISOMERS AND ANALOGUES[1]

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Earlier studies have shown that vitamin C is essential for the complete metabolism of phenylalanine and tyrosine. It has also been established that the alteration of the structure of these amino acids through the formation of simple derivatives, causes these compounds to be no longer dependent upon an adequate supply of the vitamin for their complete metabolism. To further study the interrelation between vitamin C and the metabolism of these aromatic amino acids, the various isomers and analogues were needed in optically active form. This investigation studied the synthesis and resolution of *ortho*-tyrosine, *meta*-tyrosine, 2,5-dihydroxyphenylalanine, 2,4-dihydroxyphenylalanine, and 2,3-dihydroxyphenylalanine. Also, preliminary metabolic investigation of *l* and *d* isomers of *meta*-tyrosine was undertaken using the Warburg manometric technique.

meta-Tyrosine was synthesized by a modified Erlenmeyer method which involved the reaction of hydriodic acid with the azlactone formed through the condensation of acetylglycine and *m*-hydroxybenzaldehyde. The N-formyl derivative of this amino acid formed a brucine salt one diasterioisomer of which was alcohol soluble but water insoluble. As the other diasterioisomer exhibited the inverse solubility relationships, separation of the optically active forms through repeated recrystallization was possible.

The application of the isomers of *meta*-tyrosine to metabolic study required the establishment of configuration of the *d*- and *l*-enantiomorphs isolated. This was accomplished by two methods. In the first, the procedure of Lutz and Jirgensons[2] was made use of which involved the measurement of optical activity in relation to differing concentrations of acid and base. The Lutz and Jirgensons rule states that amino acids of the natural, or *levo*, configuration show, as the ratio of moles of acid added to moles of compound present is increased, an increasing positive or a decreasing negative rotation. Thus that isomer which was isolated from the water insoluble brucine salt, and showed a decreasing negative rotation as acidity was increased, was designated as possessing the natural configuration. In the second procedure for proof of configuration the specific *d*-amino acid oxidase was obtained in purified form from kidney tissue and incubated with both isomers. The isomer which was shown to possess the *levo* configuration according to the previous procedure was not

[1] Doctoral thesis No. 831, submitted December 18, 1946.
[2] Lutz and Jirgensons. Ber. 63, 448 (1930).

attacked by the *d*-amino acid oxidase, as no keto acid production could be detected by the sensitive 2,4-dinitrophenylhydrazine reagent. The other isomer, however, gave 100 per cent of the calculated amount of keto acid, which thus established the fact that the compound possessed the *d* or unnatural configuration.

Warburg manometric studies with the *d*- and *l*-isomers of *meta*-tyrosine showed the oxygen uptake observed with normal and scorbutic tissue paralleled the uptake observed for naturally occurring tyrosine. However, the values for *l-meta*-tyrosine were consistently lower than those observed with tyrosine itself. The rates of oxygen uptake observed with *l*-tyrosine and *l-meta*-tyrosine were in sharp contrast. The *meta*-tyrosine isomer showed very rapid uptake over the first hour, but the second and third hours of incubation resulted in no additional evidence of oxidation. Tyrosine itself, on the other hand, showed a fairly constant rate of oxygen uptake over the full three hour period. The number of atoms of oxygen absorbed per mole of tyrosine substrate added did not approach the values reported for the complete oxidation of tyrosine.

The investigation of Dakin's[3] 2-methyl-4-(2′-acetoxy-benzal)-5-oxazolone as a possible starting material for the synthesis of N-acetyl-*o*-tyrosine for resolution studies showed that his supposed azlactone was 3-ketocoumarin. Through conversion of the coumarin to trans-α-acetamido-*o*-hydroxycinnamic acid and subsequent reaction of this compound with acetic anhydride, 2-methyl-4-(2′-acetoxybenzal)-5-oxazolone was synthesized. The product was shown to be different from the azlactone claimed by Dakin. Although the trans-α-acetamido-*o*-hydroxycinnamic acid was converted to the desired N-acetyl-*o*-tyrosine by catalytic reduction, the compound was found more readily available through application of the modified Erlenmeyer synthesis used in the preparation of *meta*-tyrosine. To prevent coumarin formation, the methyl ether of salicylaldehyde was used in the preparation of the azlactone intermediate. The over-all yield of amino acid obtained was 47 per cent.

The resolution of *o*-tyrosine, through the use of the N-formyl derivative according to the procedure used successfully in the isolation of the isomers of *m*-tyrosine, could not be accomplished. The rotations of the brucine salts obtained from a series of solvents were so similar as to indicate that no separation of isomers was taking place. With the N-acetyl derivative of *o*-tyrosine, however, a salt was obtained from ethyl alcohol which showed an increasing negative rotation on repeated recrystallization. From this brucine salt, after a constant rotation was reached on recrystallization, was isolated a dextrorotatory amino acid. As this amino acid showed a decreasing positive rotation as the ratio of acid was increased, it possessed the unnatural configuration. The alcohol soluble isomer could be recrystallized only from ethyl acetate. The yield of amino acid was very poor and the procedure laborious.

The synthesis of 2,5-dihydroxyphenylalanine was undertaken in view of its relationship to homogentisic acid and its postulated role as an

[3] Dakin. Jour. Biol. Chem. 82, 439 (1929).

intermediate in tyrosine metabolism. The amino acid obtained through application of the modified Erlenmeyer method previously applied, gave a product which agreed in properties with those given for the molecule by Schaaf and Labouchère[4]. The melting point given by Hirai[5] of 205° was too low as our value, in agreement with Schaaf and Labouchère, was 243°. The hydantoin of the amino acid was prepared and analysed, to further establish the nature of the product. The analytical data were in agreement with the theoretical nitrogen content. An independent synthesis of 2,5-dihydroxyphenylalanine was attempted through a procedure the first step of which involved the condensation of 2-hydroxy-5-nitrobenzyl chloride with ethyl acetamidomalonate. The ethyl α-acetamido-2-hydroxy-5-nitrobenzyl malonate obtained was converted readily through hydrobromic acid hydrolysis to 2-hydroxy-5-nitrophenylalanine. Reduction of this compound to 2-hydroxy-5-aminophenylalanine was accomplished with tin and hydrochloric acid. Diazotization and subsequent hydrolysis of the diazonium salt did not yield 2,5-dihydroxyphenylalanine. No crystalline product could be isolated in the investigation of several methods.

The modified Erlenmeyer method was successful in the conversion of 2-phenyl-4-(2′,3′-dimethoxybenzal)-5-oxazolone to 2,3-dihydroxyphenylalanine. The N-formyl derivative of this amino acid could not be resolved as the brucine salt. The reaction of hydriodic acid with 2-phenyl-4-(2′,4′-dimethoxybenzal)-5-oxazolone was exceptional in that no amino acid was obtained.

[4] Schaaf and Labouchère. Helv. chim. acta. 7, 357 (1924).
[5] Hirai. Biochem. Z. 189, 88 (1927).

SOME SUBSTITUTED 3-INDOLEACETIC ACIDS[1]

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A series of substituted derivatives of 3-indoleacetic acid, a naturally occurring plant growth hormone, was prepared in order to determine the effect of the substituents upon the plant growth activity. Chloro and nitro groups were introduced; other workers had found that these groups markedly increased the plant growth activity of phenoxyacetic and benzoic acids.

The Fischer Indole Synthesis, whereby a phenylhydrazone under the influence of an acidic reagent splits out a molecule of ammonia and forms an indole ring, was employed for the preparation of substituted acids. Cyclization was brought about by mixing a substituted phenylhydrazone with five times its weight of anhydrous zinc chloride and by heating at temperatures ranging from 100° to 170°. The product obtained in this manner was extracted with ether, and saponified with alcoholic potassium hydroxide after the ether was removed. The free acid was obtained by acidification and extraction with ether. Using this general method the following compounds were prepared.

3-(2-Methyl-7-chloroindole)-acetic acid, m.p. 164°–164.5°, was obtained in 33 per cent yield from ethyl levulinate *o*-chlorophenylhydrazone, m.p. 58.5°–59.5°, by heating at 100° for one hour.

3-(2-Methyl-5-chloroindole)-acetic acid, m.p. 190° (dec.) was prepared in 44 per cent yield from ethyl levulinate *p*-cholorophenylhydrazone, m.p. 104°–106°, by heating at 125°–135° for one hour.

3-(2-Methyl-5,7-dichloroindole)-acetic acid, m.p. 215° (dec.),* was obtained in 43 per cent yield from ethyl levulinate 2,4-dichlorophenylhydrazone, m.p. 74°–76° (dec.), by heating at 165°–170° for one hour.

A modification of the above procedure was used to prepare 3-(2-methyl-7-nitroindole)-acetic acid, m.p. 265° (dec.). Ethyl levulinate *o*-nitrophenylhydrazone, m.p. 59.5°–60°, was boiled with concentrated hydrochloric acid saturated with zinc chloride for one hour. The product was worked up in the usual manner and gave a 28 per cent yield of the acid.

3-Indoleacetic acid was prepared from glutamic acid in 30 per cent yield. Langheld's method, employing sodium hypochlorite as the oxidizing agent, was adapted for the preparation of larger quantities of β-formylpropionic acid from glutamic acid. The semi-aldehyde was converted into the phenylhydrazone. Alcoholic sulfuric acid was used to bring about ring closure.

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[1] Doctoral thesis No. 837, submitted March 19, 1947.

The plant growth activity of the compounds prepared above was determined by the Pea Test. The following results were obtained:

3-(2-Methyl-5-chloroindole)-acetic acid was active at concentrations of 20.0, 10.0, 5.00, 2.00 and 1.00 mg. per liter.

3-(2-Methyl-7-chloroindole)-acetic acid was active at concentrations of 20.0, 10.0 and 5.00 mg. per liter.

3-(2-Methyl-5,7-dichloroindole)-acetic acid was active at concentrations of 20.0 and 10.0 mg. per liter.

3-(2-Methyl-7-nitroindole)-acetic acid was inactive at the concentrations tested, 20.0, 10.0, 5.00, 2.00 and 1.00 mg. per liter.

An attempt to prepare 3-(7-nitroindole)-acetic acid from β-formylpropionic acid *o*-nitrophenylhydrazone, m.p. 155°–156° (dec.), using sulfuric acid was unsuccessful. The hydrazone, when reacted with alcoholic hydrogen chloride, gave ethyl β-formylpropionate *o*-nitrophenylhydrazone, m.p. 75°–75.5°. The ester also failed to give the desired acid when cyclized with zinc chloride.

The preparation of 3-(5-nitroindole)-acetic acid from β-formylpropionic acid *p*-nitrophenylhydrazone, m.p. 185°–186°, using alcoholic sulfuric acid, was not successful.

β-Formylpropionic acid 2,4-dichlorophenylhydrazone, m.p. 181°–182°, when treated with alcoholic hydrogen chloride, yielded a compound which melted at 215°–216° (dec.) and did not give the correct nitrogen analysis for either ethyl β-formylpropionate 2,4-dichlorophenylhydrazone or ethyl 3-(5,7-dichloroindole)-acetate. Cyclization of the hydrazone with alcoholic sulfuric acid also failed.

An attempt to prepare 3-(7-chloroindole)-acetic acid from β-formylpropionic acid *o*-chlorophenylhydrazone, m.p. 176.5°–178.5° (dec.), by cyclization with alcoholic sulfuric acid was unsuccessful.

Several compounds, which appeared to be similar to the anhydride that Fischer prepared from levulinic acid phenylhydrazone, were obtained in the course of the investigation; the corresponding derivative of β-formylpropionic acid *o*-nitrophenylhydrazone melted at 101.5°–102°, and the derivative of β-formylpropionic acid phenylhydrazone melted at 94°–95°.

THE GENERALIZED LAPLACE TRANSFORMATION WITH APPLICATIONS TO PROBLEMS INVOLVING FINITE DIFFERENCES[1]

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The generalized Laplace transformation defined by the infinite series,

$$(1) \qquad L\left\{ F(t) \right\}_E = f(s) = \sum_{n=0}^{\infty} \frac{F(n)}{s^{n+1}}, \; E^m F(t) = F(t+m),$$

has many of the advantages for the calculus of finite differences that the regular Laplace transformation has for the ordinary calculus. That is, by means of the equations,

$$(2) \qquad L\left\{ E^m F(t) \right\}_E = s^m f(s) - \sum_{k=0}^{m-1} s^{m-1-k} F(k),$$

$$(3) \qquad L\left\{ \Delta^m F(t) \right\}_E = (s-1)^m f(s) - \sum_{k=0}^{m-1} (s-1)^{m-1-k} F(k), \; \Delta = E - 1,$$

a simultaneous system of linear difference equations may be reduced to a simultaneous linear algebraic system without the introduction of extraneous constants. In general the transformation (1) will transform a class of functions into an essentially more simple class.

The necessary and sufficient condition that $F(t)$ possess a generalized Laplace transform is that it be of exponential order, $F(t) < M\alpha^t, t > T$, and is defined at all integral points. The infinite series (1) will converge uniformly for $|s| > \alpha_0 > \alpha$ and the transform $f(s)$ must be regular at infinity. Under these conditions the power series in $\frac{1}{s}$ may be differentiated term by term with respect to s so a system of difference equations with rational coefficients may be changed to a system of differential equations by the formula

$$(4) \qquad L\left\{ t^{(m)} F(t) \right\}_E = \left(-\frac{d}{ds} \right)^m s^m f(s), \; t^{(m)} = t(t-1)\ldots(t-m+1).$$

The inverse generalized Laplace transformation may be evaluated

[1] Doctoral thesis No. 857, submitted June 9, 1947.

by the usual partial fraction expansion of the transform f(s) but the complex inversion integral is more elegant. Given that

$$F(t) = L^{-1}\{f(s)\}_E \tag{5}$$

then

$$F(t) = \frac{1}{2\pi i}\int_C z^t f(z)\, dz, \tag{6}$$

where the contour of integration is a closed curve which encloses the singular points of f(s). It follows then that

$$F(t) = \text{sum of the residues of } s^t f(s). \tag{7}$$

Multiplication of the two series which define f(s) and g(s) yields the convolution summation which corresponds to the faltung integral of the regular Laplace theory:

$$L^{-1}\{f(s)g(s)\}_E = \sum_{n=o}^{t-1} F(t-n-1)G(n). \tag{8}$$

The differential equations which describe an irregular physical system may often be replaced by difference equations which lend themselves more readily to a numerical solution. The M transformation,

$$L\{F(k)\}_M = f(s) = 2\sum_{n=o}^{\infty} \frac{F(n)}{s^{n+1}} - \frac{F(O)}{s}, \tag{9}$$

is linearly related to the generalized Laplace transformation and is designed for the solution of difference equations which arise from the substitutions,

$$\begin{aligned} &x \to hk, \\ &\frac{d}{dx}F(x) \to \frac{M}{h}F(k) = \frac{F(k+1) - F(k-1)}{2h}, \\ &\frac{d^2}{dx^2}F(X) \to \frac{\Delta^2}{h^2}F(k) = \frac{F(k+1) - 2F(k) + F(k-1)}{h^2}, \end{aligned} \tag{10}$$

where h is the finite difference interval and k takes on integral values. Application of (9) yields the solution of the difference equations which are usually obtained by laborious solution of a system of simultaneous equations. The inverse transforms are best evaluated by the complex inversion integral,

$$F(k) = \frac{1}{2\pi i}\int_c \frac{z^k}{2}\left[f(z) + \frac{F(O)}{z}\right]dz, \tag{11}$$

or by the convolution summation,

$$(12)\quad L^{-1}\Big\{f(s)g(s)\Big\}_M = 2\sum_{n=o}^{k-1} F(k-n-1)G(n) - \frac{G(O)}{s}f(s) - \frac{F(O)}{s}g(s) - \frac{F(O)G(O)}{s^2}.$$

THE EFFECT OF NITROGEN FERTILIZATION ON THE NITROGEN NUTRITION OF LEGUMES[1]

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Methods heretofore employed in the study of nitrogen fixation by legumes have not been entirely satisfactory. Most have been indirect in their approach, and as a result a great deal of confusion exists in the literature on the subject. Recently the introduction and use of isotopic tracers in the study of plant and animal nutrition has greatly facilitated the acquisition of more reliable information. The employment of the stable nitrogen isotope of mass 15 has made it possible to determine the exact source of nitrogen in leguminous plants, thus giving an accurate appraisal of the fixation process.

Greenhouse experiments designed to study the effects of nitrogen fertilization on nodulation, yield, nitrogen content and nitrogen fixation by inoculated legumes were conducted over a period of 18 months. N^{15} enriched nitrogen was used in 3 experiments with soybeans and one experiment with lespedeza, sweet clover, red clover and peanuts. Ordinary nitrogen was used in experiments with sweet clover, lespedeza, red clover, white Dutch clover and peanuts. Several sources of nitrogen were used in the various experiments.

Nitrogen in the form of calcium nitrate was applied to soybeans, lespedeza, white Dutch clover, sweet clover and peanuts at the time of planting and approximately 5 weeks after planting. Sodium nitrate and ammonium sulfate at rates of 20 and 60 p.p.m. were applied to red clover at planting and 10 and 20 days after planting. Potassium nitrate was applied to soybeans and sweet clover at planting and to soybeans 5 weeks after planting in a sand-clay culture.

Plants were harvested approximately 6 and 11 weeks after emergence. The effect of nitrogen fertilization on nodulation was determined at the early harvest by counting the number of nodules per plant and noting their size and distribution on the roots. Dry weight yields and nitrogen determinations were made for both early and late harvests. Absorption of combined nitrogen and subsequent effects on fixation were measured by determining the N^{15} enrichment in all nitrogen samples from plants receiving this isotope.

The results of experiments with soybeans corroborate and extend previously reported data that nodulated soybeans do not make maximum growth when dependent solely on the fixation mechanism for their nitrogen supply. It was also established that the amount of nitrogen fixed by soybeans is related inversely to the amount of available combined

[1] Doctoral thesis No. 844, submitted June 5, 1947.

nitrogen present in the soil. Fifty p.p.m. of nitrogen applied at planting had a greater depressing effect on fixation than 25 p.p.m. applied at the same time. The same was true for midseason applications; however, both rates were less effective in depressing fixation than were applications made at planting. In all cases where nitrogen was applied the total nitrogen content of the plants was increased above that found in plants receiving no nitrogen. A greater portion of the nitrogen added at planting was recovered in the tops and roots, whereas, when the addition was made at midseason the larger portion was found in the seed. Nitrogen applied at planting greatly depressed nodulation; however, when the plants were harvested 5 weeks after emergence it was found that approximately 50 per cent of the total nitrogen content was from fixation even though a near ample supply of combined nitrogen was present.

When calcium nitrate enriched with N^{15} was applied to lespedeza it was found that the added nitrogen had no appreciable effect on fixation during the first 9 weeks of growth. However, when the plants were harvested 14 weeks after emergence all nitrogen treatments had depressed fixation. Fixation was depressed more by applications of nitrogen made at planting than by midseason applications.

Red clover and sweet clover yields and nitrogen contents were increased by nitrogen fertilizers. Red clover plants, receiving 60 p.p.m. of nitrogen at planting as ammonium sulfate with N^{15} enrichment and harvested 6 weeks after emergence, fixed more nitrogen than plants receiving no nitrogen. Nitrogen applications as sodium nitrate at planting and 20 days after planting and ammonium sulfate 20 days after planting reduced fixation. Sixty p.p.m. of nitrogen as ordinary sodium nitrate resulted in yields of dry matter superior to the 20 p.p.m. rate for all dates of application when the tops of red clover were harvested approximately 66 days after emergence. Twenty p.p.m. of nitrogen as ammonium sulfate at planting gave equally as satisfactory yields as the higher rate of sodium nitrate for all dates of application. Total nitrogen content of plants receiving the larger rate of sodium nitrate at all three times of applying were significantly better than that obtained when no nitrogen was added or when 20 p.p.m. were added. Twenty p.p.m. of nitrogen as sulfate of ammonia at planting and 60 p.p.m. at planting and 10 days after planting resulted in plants having nitrogen contents comparable to those obtained from the larger rates of sodium nitrate; whereas, the smaller rate of ammonium sulfate at 10 days after planting and both rates 20 days after planting were without significant effect on nitrogen content.

When sweet clover, receiving 15 p.p.m. of nitrogen as ordinary calcium nitrate at planting, 15 p.p.m. 5 weeks after planting, and 15 p.p.m. at planting plus 15 p.p.m. 5 weeks after planting, was harvested 11 weeks after emergence it was found that there was a net increase in nitrogen content above that found in plants receiving no nitrogen plus the added amount. This indicated that fixation had been enhanced by the added nitrogen.

White Dutch clover was adversely affected by applications of calcium

nitrate at the rates of 10 and 20 p.p.m. either at planting or 5 weeks after planting.

Peanuts responded favorably to applications of calcium nitrate. Six and 12 p.p.m. of nitrogen applied at planting and 5 weeks after planting favored early maturity of fruit, whereas, excessive amounts of combined nitrogen somewhat delayed maturity.

When potassium nitrate was added to sweet clover and soybeans in sand-clay cultures at varying calcium saturation levels nodulation of the two species was affected somewhat differently. In the case of sweet clover the nodules on plants receiving combined nitrogen were small, very numerous and distributed over the lateral roots; whereas, with soybeans there was an almost complete inhibition of nodules as a result of nitrogen applications at planting.

Fixation of nitrogen by the two plants also differed. In the case of the sweet clover fixation was depressed during the first 5 weeks of growth but at 11 weeks it was found that fixation had been greatly enhanced by nitrogen applications. Fixation by soybeans was significantly depressed at low and medium calcium levels and slightly but not significantly reduced at the high calcium level.

Employing a technique whereby the rooting and fruiting zone of peanut plants were separated, combined with the use of N^{15} it was established that nitrogen absorption takes place through the gynophores. The amount of nitrogen taken in, in this manner, was related to the amount of available nitrogen in the rooting zone. In plants having access to an ample supply of nitrogen in the root zone either in a combined form or from nodulation only 29 per cent of the nitrogen applied to the fruiting zone was recovered; whereas, when a near nitrogen starvation level existed at the rooting zone recovery from the fruiting area was in excess of 98 per cent of that added.

A COMPARISON OF SOME LABORATORY METHODS FOR THE PRODUCTION OF ANTIBODIES[1]

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The purposes of this investigation were to obtain more information regarding the nature of antibody formation and to develop a simple procedure for evoking precipitating antibodies.

Many hypotheses of the mechanism of antibody formation have been suggested. They may be divided into two general proposals. One concept is that antibody production is a synthetic function of cellular systems. The second proposal is that antibodies may be produced by altering the stereochemical and/or electrochemical properties of non-antibody proteins; no synthetic mechanism is postulated.

The following chemically modified proteins were prepared for use as antigens: 3,5-diiodo-4-hydroxybenzeneazo casein, 4-sulfobenzeneazo casein, 4-carboxybenzeneazo casein, nitro casein, guanidyl casein, guanidyl egg albumen, and the iodo and 4-arsonobenzeneazo derivatives of casein, egg albumen, and crystalline plasma albumin. The proteins were purified by reprecipitation and by dialyses against distilled water, were dried over sulfuric acid in a vacuum desiccator for seven days, and were analyzed for characteristic elements such as arsenic, iodine, nitrogen and sulfur.

Recently, there have been two reports of the production of antibodies by *in vitro* incubation of blood plasma or serum with antigenic materials. Pauling and Campbell[2] prepared antibodies *in vitro* by heating mixtures of normal globulins and antigens to 65° C., slowly cooling, and incubating for several days at 57°. The mixtures were then dialyzed against a 1 per cent solution of sodium chloride to remove excess antigen. Aliquots of the dialyzates gave weak but specific precipitin tests. Bacon[3] reported the production of antibodies to diphtheria and staphylococcus toxins by a method similar to that of Ostromuislenskii[4]. A mixture of bovine plasma and toxin (2 per cent by volume of toxin) was lyophilized, and the dried material was incubated for twelve hours at 37°. The plasma was then restored to its original volume and pH and tested for precipitin content.

In the present study, the observation of antibody formation by incubation of plasma and antigens *in vitro* was extended to the formation of precipitins for chemically modified proteins. By the use of a slight modification of Bacon's procedure, precipitins were prepared for such

[1] Doctoral thesis No. 828, submitted December 17, 1946.
[2] Pauling and Campbell, *Sci.* 95, 440 (1942); *J. Exptl. Med.* 76, 211 (1942).
[3] Bacon, *Arch. Internal Med.* 72, 581 (1943).
[4] Ostromuislenskii, *J. Russ. Phys. Chem. Soc.* 47, 263 (1915).

antigens as 3,5-diiodo-4-hydroxybenzeneazo casein, iodo casein, 4-arsonobenzeneazo casein, iodo egg albumen, and 4-arsonobenzeneazo egg albumen. Precipitins could be demonstrated in 90 per cent of the 21 preparations studied. Cross tests indicated specificity of reaction in approximately two-thirds of the preparations. The antigens containing arsenic or iodine were the most satisfactory of the antigens studied in causing production of antibodies, in regard to both strength and specificity of precipitin reactions.

In attempts to develop a rapid method for production of the relatively large amounts of antibodies which are needed for chemical studies of antibody composition, yeast was tested because of its rapid synthesis of protein as demonstrated in the manufacture of nutritional concentrates[5].

There are few reports in the literature on the production of antibodies by microorganisms. Rosenow[6] obtained substances resembling agglutinins from streptococci, pneumococci, staphylococci, and *B. subtilus* suspensions which had been autoclaved for 48 hours at 17 pounds pressure in 0.5 N acid solution. Deutschmann[7] claimed to have produced antitoxins by culturing yeast in media containing a toxin.

In the course of this study, acquired precipitins have been demonstrated in extracts of a yeast, *Saccharomyces cerevisiae,* cultured in media containing foreign proteins. The antigens tested were 4-sulfobenzeneazo casein, 4-carboxybenzeneazo casein, guanidyl casein, guanidyl egg albumen and the 4-arsonobenzeneazo and iodo derivatives of casein, egg albumen, and crystalline plasma albumin.

In the method employing *S. cerevisiae,* inocula of Strain No. 567 of the Northern Regional Research Laboratory were incubated in 100 ml. of a 15–20 per cent (g./ml.) solution of molasses for 24 hours at 30°. Aliquots of the culture containing approximately 6×10^8 cells were transferred to fresh medium (100 ml.) containing the antigen in 0.1 per cent concentration, and were incubated for 48 hours at 30°. After incubation, the cells were separated by centrifugation, washed with water, cytolyzed by grinding with sea sand and ether for approximately 30 minutes, and extracted with 25 ml. of a 5 per cent solution of sodium chloride. The extracts were clarified by centrifugation and treatment for 15–20 minutes with 0.1 g. of Filter Cel. The extracts were then tested for precipitin content by Hanks' micro method[8]. Specific precipitin reactions were observed in 82 per cent of 17 of such preparations tested.

Extracts giving macro precipitin tests were obtained from yeast cells which were transferred to fresh media containing the same concentration of antigen each 48 hours for a total of 2 to 6 transfers, and then were cytolyzed and extracted by the procedure given above. In tests of extracts of 17 of such cultures, macro tests indicated the presence of specific precipitins at selected transfer levels in 14 (82 per cent) cases.

[5] Fink, *Vorratspflege u. Lebensmittelforsch.* 1, 51, 107 (1938).
[6] Rosenow, *J. Infectious Diseases* 76, 163 (1945).
[7] Deutschmann, Brit. Patent 239, 302, June 11, 1924.
[8] Hanks, *J. Immunol.* 28, 95 (1935).

In each culture, there was an optimal number of transfers. At some other transfer levels, weaker and less specific precipitation was noted.

Considerable variation in strength and specificity of precipitin reactions was noted in micro and macro tests of yeast extracts prepared during 16 months of experimentation.

STUDIES ON OXYGEN-CARRYING COBALT COMPOUNDS[1]

VIII. Analogues of Disalicylalethylenediimine Cobalt with o-Hydroxycarbonyl Compounds Other Than Salicylaldehyde

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In Paper IX of this series it will be shown that substitution in or for the ethylenediamine portion of the molecule of the oxygen-carrying compound bi-(disalicylalethylenediimine)-μ-aquo-dicobalt yielded cobalt derivatives which invariably failed to absorb and release oxygen reversibly. As indicated in Papers V, VI, and VII dealing with the derivatives from 3-nitrosalicylaldehyde, 3-methoxysalicylaldehyde and various higher 3-alkoxysalicylaldehydes, substitution in the salicylaldehyde portion of the molecule is permissible, indeed beneficial in that the substituted derivatives may have a higher rate of oxygenation, lower deoxygenation temperature, and other desirable physical properties.

The present paper deals with a variety of substituted salicylaldehydes and other β-diketones analogous to salicylaldehyde. Most of the cobalt-ethylenediamine compounds of these β-dicarbonyl compounds were inactive. The few which were active absorbed oxygen slowly and failed to absorb the theoretical amount of oxygen.

The method and conditions under which the cobalt compound was formed appeared to be of great importance in determining if the material was to absorb oxygen. Consequently the method used in making each of the cobalt compounds is reported in some detail even at the risk of what might appear to be needless repetition.

Some generalizations may be drawn from this work regarding substitution. Apparently large alkyl substituents, greater than ethyl, cannot be introduced and an active product obtained; this is true for substituents both on the ring and on the aldehyde group (phenones). Not more than one substituent, alkyl, nitro, chloro, bromo, or alkoxy may be introduced. The substitution in the 3- position is favored in preference to the others but not invariably.

ALKYL SUBSTITUENTS

The four possible methyl substituted salicylaldehydes, were synthesized from *ortho-*, *meta-*, and *para*-cresol by the Reimer-Tiemann reaction. The Schiff's base of each of these aldehydes with ethylenediamine was prepared and the cobalt derivative of each of these Schiff's bases. The

[1] Preceding parts of this discussion were published in this Journal as follows: Papers I, II, and III, Vol. XXI, pp. 271–309, 1947; Papers IV, V, VI, and VII, Vol. XXI, pp. 311–349, 1947.

cobalt compounds were also prepared by the direct interaction of the aldehyde, diamine, and cobalt salt in dilute alcohol.

Di-(2-hydroxy-3-methylbenzal)-ethylenediimine cobalt was prepared by the direct interaction method and also through the potassium salt of the Schiff's base. Neither of these preparations absorbed oxygen when placed in an atmosphere of oxygen at 200 pounds pressure.

Di-(2-hydroxy-4-methylbenzal)-ethylenediimine cobalt, when prepared by the direct interaction method, showed no oxygen-carrying activity. A preparation of the compound made by boiling an aqueous suspension of a mixture of the Schiff's base and a cobalt salt was active, reversibly absorbing and releasing 1.56 per cent oxygen. It did not absorb oxygen from air at atmospheric pressure and was therefore less active than the parent compound, disalicylalethylenediimine cobalt.

Di-(2-hydroxy-5-methylbenzal)-ethylenediimine cobalt was synthesized by both methods. The compound prepared by the direct interaction method carried 2.7 per cent oxygen. This compound did not absorb oxygen from the air at atmospheric pressure and was therefore less active than the parent compound. A preparation of the material through the Schiff's base was inactive.

Di-(2-hydroxy-6-methylbenzal)-ethylenediimine cobalt was prepared by the direct interaction method and was found to have an oxygen-carrying capacity of 2.34 per cent.

The work on this series of compounds was handicapped by the low yields obtained in the Reimer-Tiemann reaction on the various cresols. None of the preparations closely approached the theoretical oxygen-carrying capacity, and the active preparations failed to absorb oxygen from the air at atmospheric pressure so that these compounds appear to be of little interest. The failure to obtain an active compound with the 3-methyl aldehyde should not be regarded as conclusive. With sufficient aldehyde available, it might be possible to find conditions which would lead to an active compound. Apparently the oxygen-carrying property resides in a peculiar crystal structure and the method of formation has considerable to do with the crystal form obtained. Four dimethylsalicylaldehydes were prapared: 3,5-, 3,6-, 4,5-, and 4,6- by means of the Duff reaction on the requisite phenol. The Schiff's bases of these aldehydes with ethylenediamine were made, and the cobalt derivatives of the latter were prepared. None of the compounds were active toward oxygen.

One ethylsalicylaldehyde was prepared, 2-hydroxy-5-ethylbenzaldehyde. Its Schiff's base was crystalline and the cobalt derivative carried 3.94 per cent oxygen. Its rate of oxygenation was about that of the parent compound.

Several more highly, alkyl substituted salicylaldehydes were prepared and their ethylenediamine-cobalt derivatives studied:

2-hydroxy-3-methyl-5-*tert*-butylbenzaldehyde
2-hydroxy-4-methyl-5-*tert*-amylbenzaldehyde
2-hydroxy-3-*iso*-propyl-6-methylbenzaldehyde
2-hydroxy-3-*tert*-amylbenzaldehyde

None of the cobalt compounds carried oxygen.

The compound, formylcamphor, contains a keto group adjacent to an aldehyde group and has been shown to enolize yielding a hydroxyl group adjacent to the aldehyde group. The enol structure is similar to that found in aromatic o-hydroxyaldehydes. A nickel derivative of the Schiff's base of formylcamphor with ethylenediamine was prepared by Pfeiffer and Mitarb (9), but no other metal derivatives appear to have been reported. The cobalt derivative was prepared without difficulty. It was rose-red in color and melted with decomposition. It failed to absorb oxygen on being subjected to the usual tests. The cobalt derivatives of phenylenediamine and formylcamphor was also inactive.

HYDROXY AND METHOXY SUSTITUENTS

2,3-Dihydroxybenzaldehyde and 2,4-dihydroxybenzaldehyde yielded crystalline Schiff's bases with ethylenediamine. The cobalt derivatives of these bases were prepared and found to be inactive. 2-Hydroxy-5-methoxybenzaldehyde and 2-hydroxy-5-ethoxybenzaldehyde were synthesized from p-methoxyphenol and p-ethoxyphenol respectively by the Duff reaction. Their Schiff's bases with ethylenediamine were also yellow, crystalline materials. The cobalt derivatives were also inactive.

CHLORO AND BROMO SUBSTITUENTS

3-Chlorosalicylaldehyde was obtained from o-chlorophenol by the Duff reaction. Its Schiff's base with ethylenediamine yielded a red-brown cobalt compound which carried 1.8 per cent oxygen. The rate of absorption was very low, however.

3-Bromo and 5-bromosalicyladehyde yielded cobalt compounds which were inactive. Four aldehydes prepared from commercially available phenols by the Duff reaction, 2-hydroxy-3-chloro-5-*tert*-butylbenzaldehyde, 2-hydroxy-5-chloro-6-methylbenzaldehyde, 2-hydroxy-3-*iso*-propyl-5-chloro-6-methylbenzaldehyde, and 2-hydroxy-3-bromo-5-*tert*-butylbenzaldehyde, were also studied. All of the cobalt compounds were inactive.

NITRO SUBSTITUENTS

Although the cobalt-ethylenediamine derivatives of 2-hydroxy-3-nitrobenzaldehyde (3-nitrosalicylaldehyde) was found to be an oxygen carrier (Paper V), that from 2-hydroxy-5-nitrobenzaldehyde was found to be inactive. The cobalt derivative was made by two different methods and was heated at 170° in a vacuum before testing it for activity toward oxygen. The derivatives of 2-hydroxy-3-nitro-5-methylbenzaldehyde and of 2-hydroxy-3-methoxy-5-nitrobenzaldehyde were also inactive.

o-HYDROXYPHENONES

When properly prepared the cobalt compound of o-hydroxyacetophenone and ethylenediamine, di-(2-hydroxyacetopheno)-ethylenediimine cobalt, was found to carry oxygen reversibly to the extent of about 4 per cent. The rate of oxygenation was very low, however, even under relatively high oxygen pressure. Since the introduction of a nitro group into the 3-position of the parent compound, disalicylalethylenediimine

cobalt, greatly increased the rate of oxygenation, the derivative of 2-hydroxy-3-nitroacetophenone was also synthesized. The compound was inactive.

2-Hydroxy-4-methylacetophenone, 2-hydroxy-5-methylacetophenone, 2,4-dihydroxyacetophenone (resacetophenone) and 2-hydroxypropiophenone were also studied; all yielded crystalline Schiff's bases with ethylenediamine, and all of the Schiff's bases yielded cobalt derivatives without difficulty. The compounds from 2-hydroxy-4-methylacetophenone and 2-hydroxypropiophenone were inactive. That from 2-hydroxy-5-methylacetophenone carried 1 per cent oxygen, while that from 2,4-dihydroxyacetophenone carried 0.3 per cent oxygen after drying in a vacuum at 100° but after being heated to 170° in a vacuum was inactive.

EXPERIMENTAL WORK

GENERAL NOTE ON THE REIMER-TIEMANN REACTION

Tiemann and Schotten (1) described the preparation of *meta-*, *ortho-*, and *para*-cresols from the corresponding toluidines and the preparation of all of the isomeric homosalicylaldehydes (methylsalicylaldehydes) resulting from the application of the Reimer-Tiemann reaction on these cresols. All the later references to the preparation of those compounds are based on the original article cited above. During the course of the work being reported here, the preparation of these homologues was repeated several times, and the improvements made in the synthesis of these particular aldehydes justifies including a detailed account of the work even in view of the considerable general literature on the Reimer-Tiemann reaction.

The literature contains numerous references, mostly patents, to methods of separating the three isomeric cresols and to methods for the quantitative determination of one isomer in the presence of the others. However, no good laboratory procedure was found for purifying a product already 90 per cent pure, and the following work was done on technical grade cresols carefully fractioned in vacuum.

2-HYDROXY-5-METHYLBENZALDEHYDE

p-Cresol, Eastman Kodak Company P-449, a black liquid at room temperature, was fractionated under vacuum. The product was colorless and crystallized upon standing at room temperature; m.p.: 35°, reported m.p.: 36°. A mixture of 140 g. of potassium hydroxide, 45 g. of *p*-cresol, and 300 ml. of water was heated just to boiling in a 1 l. flask fitted with a reflux condenser. By means of a dropping funnel 80 g. of chloroform was added at such rate that the reaction mixture boiled gently. This addition required about 45 to 60 minutes, during which the color gradually changed from yellow to orange and to dark red. The reaction mixture was then refluxed for 3 hours and then acidified with 175–200 ml. of concentrated hydrochloric acid. The mixture was distilled until the distillate was clear. A yellow oil separated from the milky distillate.

The distillate was extracted with one 100 ml. portion and two 50 ml.

portions of ether. The ether extract was then shaken with two 100 ml. portions of a 10 per cent sodium bisulfite solution, followed by a third extraction with the same volume of a warm 20 per cent bisulfite solution, and then with 25 ml. of distilled water.

The bisulfite extract was then placed in a flask connected to a steam generator and fitted with a dropping funnel. A suction flask was used for a receiver and was connected to a water aspirator and a gentle suction applied to carry away the sulfur dioxide gas formed in the ensuing reaction. Fifty ml. of concentrated sulfuric acid was added through the dropping funnel. After the evolution of sulfur dioxide had subsided the solution was steam distilled. The 2-hydroxy-5-methylbenzaldehyde was collected in the receiver as a light yellow oil which crystallized readily on cooling. It was filtered off. An ether extract of the filtrate showed but a trace of the aldehyde and hence further extraction was not necessary. The solution remaining from the bisulfite extraction was evaporated to remove ether, and the remaining cresol was recovered.

The 2-hydroxy-5-methylbenzaldehyde was recrystallized by dissolving it in a minimum of hot ethyl alcohol, then adding about an equal volume of distilled water, and heating until the milkiness disappeared leaving a clear solution. The solution was then allowed to cool to room temperature, whereupon beautiful, glistening, white plates were obtained. The solution was cooled in ice, filtered, washed on the filter with a small portion of ice-cold ethyl alcohol, and dried by suction in air. Yield: 15 per cent; m.p. crude product: 52°–53°; after first recrystallization: 54°; after second recrystallization: 55.8°; reported by Tiemann and Schotten (1): 56°.

A lower yield was obtained when the reaction mixture was allowed to stand a considerable length of time (overnight or weekend) before acidification and steam distillation. This may be due to polymerization of the aldehyde when remaining in contact with the concentrated alkali.

This aldehyde was also synthesized by the Duff reaction (5,6); yield: 30 per cent; m.p.: 54°.

DI-(2-HYDROXY-5-METHYLBENZAL)-ETHYLENEDIIMINE

To 4.3 g. (0.05 mole) of 68.7 per cent ethylenediamine dissolved in 200 ml. of water was added 18.6 g. of 2-hydroxy-5-methylbenzaldehyde dissolved in 400 ml. of 95 per cent ethyl alcohol warmed to 60°. A yellow precipitate formed immediately which was filtered off and recrystallized once from 95 per cent ethyl alcohol and twice from a mixture of benzene and petroleum ether; m.p.: 164°.

DI-(2-HYDROXY-5-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

A quantity of 4.3 g. of ethylenediamine (68.7 per cent, 0.05 mole), was dissolved in 100 ml. of 50 per cent alcohol-water solution, and added to 12.45 g. (0.05 mole) of cobalt acetate, $Co(C_2H_3O_2)_2 \cdot 4H_2O$, dissolved in 150 ml. of 50 per cent ethyl alcohol at 50°. A solution of 13.6 g. (0.1 mole) of 2-hydroxy-5-methylbenzaldehyde and 2 g. of sodium acetate dissolved in 200 ml. of 95 per cent alcohol was added to the cobalt-diamine solution.

The volume of solvent used was probably a little large as only a small amount of the violet-brown precipitate formed. After being shaken for 15 minutes the mixture was filtered, washed with cold, 50 per cent alcohol, and sucked dry with a sheet of thin rubber covering the precipitate in the funnel.

This violet-brown material was dried for 4 hours at 100° in a vacuum, then pulverized, yielding a fluffy, reddish-brown powder, and dried to constant weight in a vacuum at 100°.

A weighed sample of the material, placed in an atmosphere of oxygen at 200 pounds pressure became much darker brown in color, increased considerably in volume, and gained in weight 2.7 per cent, owing to the absorption of oxygen. It was readily deoxygenated in a vacuum at room temperature or at 100° at atmospheric pressure. The oxygenation cycle was repeated several times, but the oxygen-carrying capacity did not increase. On exposure to air at atmospheric pressure the material did not absorb oxygen.

A preparation of this cobalt compound made by dissolving the Schiff's base, di-(2-hydroxy-5-methylbenzal)-ethylenediimine, and two equivalents of sodium hydroxide in water and treating with the correct amount of cobalt acetate in a solution containing a little acetic acid yielded a brown precipitate which on drying at 100° in a vacuum decomposed slowly and continuously liberating 2-hydroxy-5-methylbenzaldehyde. The product did not absorb oxygen.

The failure to obtain a satisfactory oxygen-carrying product in this manner is puzzling as this method of preparation is the best in the case of the parent and other oxygen-carrying compounds.

2-HYDROXY-4-METHYLBENZALDEHYDE AND 2-HYDROXY-6-METHYLBENZALDEHYDE

Tiemann and Schotten (1) reported one homosalicylaldehyde from the Reimer-Tiemann reaction on *m*-cresol. Later Chuit and Bolsing (2) isolated the expected two aldehydes and gave several possible methods for the separation of the isomers. The procedure of Chuit and Bolsing was followed in the preparation and separation of these two aldehydes.

m-Cresol, Eastman Kodak Company P-369, was fractionated under a vacuum, and a water-clear product was obtained; m.p.: 11°; reported m.p.: 11°–12°. Both the purified and the practical grade were used with about equal success.

A mixture of 260 g. of *m*-cresol, 1,399 g. of sodium hydroxide, and 75 g. of potassium hydroxide was dissolved in 1,000 ml. of water in a 5 l., three-necked flask fitted with a motor-driven stirrer, reflux condenser, and a dropping funnel. To the above solution was added gradually by means of a dropping funnel 248 ml. of chloroform, the addition requiring about 90 minutes. The reaction mixture was then refluxed for an additional 90 minutes and then cooled, acidified with cold dilute sulfuric acid (1:1), and steam distilled. The yellow oil which separated from the

distillate, containing the two isomeric aldehydes and unreacted cresol, was extracted with ether. The ether solution was extracted with a sodium bisulfite solution and the latter steamed distilled after acidifying just as described above in the preparation of 2-hydroxy-5-methylbenzaldehyde. A yield of 85 g. of the mixed aldehydes was obtained from two runs using 260 g. of *m*-cresol in each.

A mixture of 82 g. of the mixed isomeric aldehydes, 3.9 g. of sodium carbonate and 200 ml. of water was steam distilled until more yellow oil failed to separate from the distillate. The yellow oil which separated was isolated by means of a separatory funnel. The carbonate solution was then acidified with dilute sulfuric acid and the steam distillation continued. The second yellow oil separated upon cooling the steam distillate.

The first oil obtained could not be induced to crystallize by cooling in an ice-salt mixture. Probably this material contained a small amount of the other isomer which lowered its melting point sufficiently to make crystallization difficult. The phenylhydrazone of this aldehyde was prepared and recrystallized twice from 95 per cent alcohol; m.p.: 168°; reported by Anselmino (3): 172° for the phenylhydrazone of 2-hydroxy-6-methylsalicylaldehyde.

The second oil crystallized on cooling and was identified as 2-hydroxy-4-methylbenzaldehyde; m.p. (recry. from dilute alcohol): 59°–60°; reported (2,3): 60–61°. The phenylhydrazone was prepared and recrystallized from alcohol; m.p.: 160°, reported (3): 161°.

A mixed melting point of the phenylhydrazones of these two aldehydes melted at 134°. This leaves no question that a separation was accomplished.

DI-(2-HYDROXY-4-METHYLBENZAL)-ETHYLENEDIIMINE

Condensation of the aldehyde and ethylenediamine proceeded readily in alcohol. The Schiff's base was recrystallized from alcohol; m.p.: 194°.

DI-(2-HYDROXY-4-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

To a solution containing 3.1 g. of cobalt acetate, $Co(C_2H_3O_2)_2 \cdot 4H_2O$, and 1.1 g. of ethylenediamine in 100 ml. of 40 per cent alcohol was added a solution of 3.4 g. of 2-hydroxy-4-methylbenzaldehyde in 50 ml. of alcohol. A light brown precipitate formed in about a minute, and the mixture was shaken frequently for about 20 minutes and then filtered on a Buchner funnel, and washed with 25 ml. of 50 per cent alcohol. The product was dried at 100° in a vacuum for 3 hours, then pulverized. There was no gain in weight or change in color when subjected to oxygen at 200 pounds pressure. A sample of the compound was heated to 170° under a vacuum but was still inactive toward oxygen.

A second preparation was made by grinding together 2.9 g. of the Schiff's base and 2.5 g. of cobalt acetate with a little water. The paste was washed into a beaker, diluted to 200 ml. and heated to boiling. The yellow slurry gradually changed to a dark brown precipitate during the heating. The beaker and contents were allowed to cool somewhat and

then filtered on a Buchner funnel. The precipitate was dried for 4 hours at 100° under a vacuum, pulverized, and redried at 100° for 1 hour. After being heated at 170° in a vacuum for 2 hours, the compound carried 1.56 per cent oxygen. It was less active than the parent compound as it did not oxygenate in air at atmospheric pressure.

DI-(2-HYDROXY-6-METHYLBENZAL)-ETHYLENEDIIMINE

This Schiff's base was prepared from the liquid aldehyde described above and ethylenediamine by condensation in alcohol; m.p. after recrystallization from alcohol: 139°.

DI-(2-HYDROXY-6-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

A solution of 6.8 g. of 2-hydroxy-6-methylbenzaldehyde in 25 ml. of warm 40 per cent alcohol was added to a solution of 6.2 g. of cobalt acetate, $Co(C_2H_3O_2)_2 \cdot 4H_2O$, and 2.2 g. of ethylenediamine in 125 ml. of 40 per cent alcohol. The mixture was shaken and a heavy brown precipitate formed immediately. After standing for 30 minutes, the bulky precipitate was filtered on a Buchner funnel and washed with cold 40 per cent alcohol. The material was dried at 100° under a vacuum for 3 hours, pulverized, and dried again for 1 hour. A weighed sample was dried to constant weight at 100° under a vacuum and then placed in an atmosphere of oxygen at 200 pounds pressure. The compound turned from red to black and absorbed 2.34 per cent oxygen. It was deoxygenated at 100°. The deoxygenated form did not gain in weight upon standing in a desiccator and therefore was not as active as the parent compound. Another sample of the same preparation dried at 170° in a vacuum had an oxygen-carrying capacity of 2.2 per cent.

2-HYDROXY-3-METHYBENZALDEHYDE

This aldehyde was synthesized from *o*-cresol by the Reimer-Tiemann reaction as described under the 5-methylaldehyde above, with the exception that the amount of sodium hydroxide used was decreased somewhat. The yield was very poor and the yellow oil obtained could not be induced to crystallize; reported m.p.: 17°. The phenylhydrazone derivative was prepared; m.p.: 93°, reported (4): 97°.

DI-(2-HYDROXY-3-METHYLBENZAL)-ETHYLENEDIIMINE

Two equivalents of 2-hydroxy-3-methylbenzaldehyde and one of ethylenediamine were condensed in ethyl alcohol yielding a bright yellow Schiff's base, m.p.: 111°. The potassium salt of this base was also prepared.

DI-(2-HYDROXY-3-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

To a solution of 6.8 g. of 2-hydroxy-3-methylbenzaldehyde dissolved in 25 ml. of 50 per cent alcohol warmed to 60° was added a solution of 6.2 g. cobalt acetate, $Co(C_2H_3O_2)_2 \cdot 4H_2O$, and 2.2 g. of ethylenediamine in 125 ml. of 50 per cent alcohol. A brown precipitate formed in a few

minutes. The flask was frequently shaken for 15 minutes, and the precipitate was filtered, washed with cold 40 per cent alcohol and dried for 3 hours at 100° under a vacuum. The material was pulverized and again dried. The product did not gain in weight or change in color when placed in oxygen at 200 pounds pressure. A second sample after being heated at 170° in a vacuum was inactive toward oxygen.

In a second preparation of this cobalt compound 5 g. of the Schiff's base and 1 g. of sodium acetate were dissolved in 150 ml. of water at 90° and treated with a solution of 2.5 g. of cobalt sulfate, $CoSO_4 \cdot 7H_2O$, dissolved in 50 ml. of warm water. A brown precipitate formed which was filtered on a Buchner funnel and washed with 100 ml. of cold water. The compound was dried at 100°. This material and a portion of it heated to 170° in vacuum did not increase in weight in oxygen at 200 pounds pressure.

2-HYDROXY-3-NITRO-5-METHYLBENZALDEHYDE

2-Hydroxy-5-methylbenzaldehyde, m.p.: 54°, was nitrated in acetic acid; m.p.: 139°; reported by Borsche (7): 141°.

DI-(2-HYDROXY-3-NITRO-5-METHYLBENZAL)-ETHYLENEDIIMINE

The condensation was effected in absolute alcohol on warming. Orange-yellow crystals; m.p.: above 225°. When treated with alkali this material yielded a blood red solution from which the orange-yellow Schiff's base was precipitated on acidifying. The range of pH over which the material changed color was 5.3 to 7.5, the color change being similar to that of methyl orange.

DI-(2-HYDROXY-3-NITRO-5-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

Because of the insolubility of the Schiff's base in hot, ethyl alcohol, the cobalt derivative was prepared from a solution of the sodium salt of the base. To a solution of 10 g. of the Schiff's base and 2 g. of sodium hydroxide in 150 ml. of water, a blood red solution, was added a solution of 10 g. of hydrated cobalt chloride and 5 ml. of acetic acid in 50 ml. of warm water. The mixture was stirred and digested for 15 minutes on the hot plate. After standing 2 hours the precipitate was filtered and washed with hot water. This dark red material was dried in a vacuum at 100°, 120°, 150°, and 175° but failed to gain in weight or change in color when subjected to oxygen at 200 pounds pressure.

A second preparation of this cobalt derivative was made using hot normal propyl alcohol in which the Schiff's base is sufficiently soluble. The product was similar in appearance and inactive toward oxygen.

DI-(2-HYDROXY-3,5-DIMETHYLBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared by dissolving the Schiff's base, m.p.: 132.5° (6), in hot, dilute sodium hydroxide and adding a hot, aqueous solution of cobalt chloride. A dark red material was obtained which was inactive toward oxygen.

DI-(2-HYDROXY-3,6-DIMETHYLBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base, di-(2-hydroxy-3,6-dimethylbenzal)-ethylenediimine; m.p.: 182° (6), was dissolved in hot ethyl alcohol and a hot, aqueous solution of cobalt acetate added. An orange-red compound was obtained which was washed with dilute alcohol and dried in a vacuum at 120°. Found: 14.90, 14.98 per cent Co; calculated: 14.98 per cent Co. This material was heated to 175° but was inactive toward oxygen.

DI-(2-HYDROXY-4,5-DIMETHYLBENZAL)-ETHYLENEDIIMINE COBALT

To 10 g. of di-(2-hydroxy-4,5-dimethylbenzal)-ethylenediimine, m.p.: 212°–214° (6), dissolved in 50 ml. of water containing 1.5 g. of sodium hydroxide was added a hot solution of 8 g. of cobalt acetate in water. The compound obtained was red in color. The compound was not active towards oxygen.

DI-(2-HYDROXY-4,6-DIMETHYLBENZAL)-ETHYLENEDIIMINE

2-Hydroxy-4,6-dimethylbenzaldehyde was synthesized from 3,5-xylenol by the Duff reaction; yield: 11 per cent; m.p.: 48°, reported: 48°–49° (8); m.p. phenylhydrazone (from dilute alcohol): 126°; reported: 126.5°–127° (8). This aldehyde and ethylenediamine were condensed in absolute alcohol; m.p.: 152°–153°.

DI-(2-HYDROXY-4,6-DIMETHYLBENZAL)-ETHYLENEDIIMINE COBALT

To 15 g. of di-(2-hydroxybenzal)-ethylenediimine in 250 ml. of hot 95 per cent alcohol was added 350 ml. of a hot, aqueous solution, containing 12 g. of cobalt acetate. The compound obtained was red in color rather than the maroon tint usually associated with oxygen-carriers. After drying and activation at temperatures up to 175° the compound was found to be inactive towards oxygen.

DI-(2-HYDROXY-5-ETHYLBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared by the direct mixing method in 50 per cent alcohol. A hot, aqueous solution containing 4 g. of cobalt acetate and 2 g. of 60 per cent ethylenediamine was added to 6 g. of 2-hydroxy-5-ethylbenzaldehyde (6) dissolved in alcohol. A brown compound precipitated immediately. This material was filtered off and dried in a vacuum at 115° for 1 hour. The compound absorbed 2.5 per cent oxygen in oxygen at 200 pounds pressure and turned dark in color. Upon activation at 175° the oxygen capacity increased to 3.94 per cent. The compound absorbed oxygen slowly from the air at room temperature.

DI-(2-HYDROXY-3-METHYL-5-*tert*-AMYLBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base di-(2-hydroxy-3-methyl-5-*tert*-amylbenzal)-ethylenediimine, m.p.: 90° (6), was dissolved in a hot, dilute sodium hydroxide solution. To this was added a hot, aqueous solution of cobalt chloride. The compound precipitated was brick red in color but was inactive towards oxygen even after being heated to 170° in a vacuum.

DI-(2-HYDROXY-4-METHYL-5-*tert*-BUTYLBENZAL)-ETHYLENEDIIMINE COBALT

The aldehyde was synthesized from 3-methyl-4-*tert*-butylphenol by the Duff reaction (6) and may well actually be the other possible aldehyde, 2-hydroxy-5-*tert*-butyl-6-methylbenzaldehyde, rather than the 2-hydroxy-4-methyl-5-*tert*-butylbenzaldehyde indicated, or even a mixture of the two. B.p. of the aldehyde: 109°–110°/2 mm., $n^{25°}$: 1.540. M.p. of the Schiff's base with ethylenediamine: 165°. The Schiff's base was only slightly soluble in alcohol or dilute sodium hydroxide, and therefore the cobalt derivative was prepared by digesting a finely powdered suspension of the Schiff's base with a solution of cobalt acetate. An orange-red compound was obtained which was subjected to the usual tests and found to be inactive.

DI-(2-HYDROXY-3-*iso*-PROPYL-6-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base, di-(2-hydroxy-3-*iso*-propyl-6-methylbenzal)-ethylenediimine, m.p.: 112°–113° (6), was dissolved in a hot, dilute sodium hydroxide solution, and to it was added a hot, aqueous solution of cobalt acetate. A red compound was obtained. The compound was also prepared by adding a hot, aqueous solution containing the required amounts of cobalt acetate and ethylenediamine to a solution of the aldehyde in alcohol. A darker red compound was obtained by this method. The compounds obtained by both methods were inactive towards oxygen.

DI-(2-HYDROXY-3-*tert*-AMYLBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base, di-(2-hydroxy-3-*tert*-amylbenzal)-ethylenediimine, m.p.: 109.5° (6) was dissolved in hot, dilute sodium hydroxide and the solution treated with a hot, aqueous solution of cobalt acetate containing a little acetic acid. A dark red compound was obtained which was not active towards oxygen.

DI-(FORMYLCAMPHOR)-ETHYLENEDIIMINE

Formylcamphor was prepared by the method of Bishop, Claisen, and Sinclair (10). M.p.: 72°–74°; m.p. of material vacuum distilled: 80°; reported (10): 70°–76°. The condensation with ethylenediamine was effected in hot methyl alcohol. The product was slightly yellow in color; m.p.: 215°; reported (9): 215°.

DI-(FORMYLCAMPHOR)-ETHYLENEDIIMINE COBALT

To 25 g. of the Schiff's base dissolved in 150 ml. of hot methyl alcohol was added a solution of 10 g. of cobalt acetate in 25 ml. of warm water. The mixture was digested 3 hours on a steam bath, then filtered and washed well with hot water. The product was dried in a vacuum at 100°. It was rose colored and melted at 190°–200° with decomposition. The material was inactive toward oxygen even after being further heated to 120° and 150° in a vacuum.

DI-(FORMYLCAMPHOR)-PHENYLENEDIIMINE

The condensation of formylcamphor and phenylenediamine was effected by bringing together warm alcohol solutions of the two. Water was added to the mixture until a slight turbidity was produced, and the solution was allowed to cool slowly. The yellow, crystalline Schiff's base which separated was filtered off, washed with water, and recrystallized from 50 per cent alcohol; m.p.: 125°.

DI-(FORMYLCAMPHOR)-PHENYLENEDIIMINE COBALT

The cobalt derivative of formylcamphorphenylenediimine was prepared by adding 5 g. of an aqueous cobalt acetate solution to a methyl alcohol solution containing 5 g. of the Schiff's base. A dark brown crystalline precipitate resulted which was dried in a vacuum at 100°. This material showed no change in color or gain in weight in oxygen at 175 pounds pressure. A sample was heated to 140°, but the material was still inactive.

DI-(2,3-DIHYDROXYBENZAL)-ETHYLENEDIIMINE

A sample of 2,3-dihydroxybenzaldehyde was obtained from the Monsanto Chemical Company; m.p.: 104°–106°; b.p.: 132°–135°/33 mm. Alcohol solutions of the necessary amounts of the aldehyde and ethylenediamine were mixed, yielding a red solution from which yellow crystals separated on cooling. After recrystallization from alcohol, this material decomposed at about 220° without melting.

DI-(2,3-DIHYDROXYBENZAL)-ETHYLENEDIIMINE COBALT

To a solution of 3 g. of the Schiff's base, 0.80 g. of sodium hydroxide and 0.04 g. of sodium acetate in 200 ml. of water, was added a solution of 2.37 g. of cobalt chloride in 20 ml. of water. An olive-colored precipitate formed which was filtered off, washed with water, and dried in a vacuum first at 100° and then at 170°. It was inactive toward oxygen. It was necessary to cool the material completely to room temperature before exposing it to air as otherwise spontaneous combustion occurred accompanied by a considerable display of fireworks.

Other preparations of this compound made by digesting together in suspension the Schiff's base and cobalt salt without alkali and by first preparing the pyridine addition product and depyridinating it were also inactive.

2,4-DIHYDROXYBENZALDEHYDE

This aldehyde was prepared by the Duff reaction (5,6). The reaction mixture after acidification with sulfuric acid was steam distilled to remove the dialdehyde as done earlier by Tiemann and Lewy (11). Only a small amount of the dialdehyde was obtained; m.p.: 127°; reported (11): 127°. The residue from the steam distillation was boiled down to a thick, red syrup which was cooled and extracted with benzene. The benzene extract

yielded white crystals of the aldehyde; m.p.: 135°–137°; reported (11): 134°–135°. Yield from 50 g. of resorcinol: 1.5 g.

DI-(2,4-DIHYDROXYBENZAL)-ETHYLENEDIIMINE

The aldehyde and the requisite amount of ethylenediamine were condensed in alcohol. Orange-yellow crystals; m.p.: above 210° (with decomp.).

DI-(2,4-DIHYDROXYBENZAL)-ETHYLENEDIIMINE COBALT

To a solution of 3 g. of the Schiff's base and 0.8 g. of sodium hydroxide in 50 ml. of water was added 2.4 g. of cobalt chloride. The brown precipitate which separated was filtered off, washed, and dried in a vacuum at 100°. It was inactive toward oxygen. A second preparation of the material was attempted in a 60 per cent alcohol solution but no precipitate formed.

2-HYDROXY-5-METHOXYBENZALDEHYDE

This aldehyde was synthesized from *p*-methoxyphenol, m.p.: 51.5°–52.5°, by the Duff reaction (5,6); m.p.: 5°; reported (5): 5°.

DI-(2-HYDROXY-5-METHOXYBENZAL)-ETHYLENEDIIMINE

The aldehyde and the required amount of ethylenediamine were condensed in alcohol; yellow crystals; m.p.: 161°.

DI-(2-HYDROXY-5-METHOXYBENZAL)-ETHYLENEDIIMINE COBALT

To a solution of 3.3 g. of the Schiff's base and 0.4 g. of sodium hydroxide in 25 per cent alcohol was added 2.4 g. of cobalt chloride. After digestion on the steam bath for 1 hour, the dark red precipitate was filtered off, washed with water, and dried in a vacuum first at 100° and then at 170°. It was inactive toward oxygen.

2-HYDROXY-5-ETHOXYBENZALDEHYDE

This aldehyde was prepared from *p*-ethoxyphenol, m.p.: 65°–66°, by the Duff reaction (5,6); yield: 16 per cent; m.p.: 48°–49°; reported (13): 51.5°.

DI-(2-HYDROXY-5-ETHOXYBENZAL)-ETHYLENEDIIMINE

The aldehyde and ethylenediamine were condensed in alcohol; yellow crystals; m.p.: 150°–152°.

DI-(2-HYDROXY-5-ETHOXYBENZAL)-ETHYLENEDIIMINE COBALT

The solubility of the Schiff's base in alcohol was so low that the cobalt derivative could not be made to crystallize from the volume necessary to dissolve the Schiff's base. Sodium hydroxide was therefore used to assist in the solution of the Schiff's base. To 3.56 g. of the Schiff's base and 0.4 g. of sodium hydroxide dissolved in 200 ml. of 25 per cent alcohol and heated to boiling was added 2.91 g. of cobalt nitrate. The mass was digested on a steam plate over night. The light-brown precipitate which formed

was filtered off, washed, and dried in a vacuum at 130°. It was inactive toward oxygen.

DI-(2,3-DIHYDROXY-5(6?)-*tert*-BUTYLBENZAL)-ETHYLENEDIIMINE COBALT

The aldehyde was obtained from *p-tert*-butylcatechol by the Duff Reaction (5,6); it may be either the 5-*tert*- or the 6-*tert*-butyl or possibly a mixture of the two. M.p.: 85°; m.p. of the phenylhydrazone: 160°–161°; m.p. of the Schiff's base with ethylenediamine: 226°. Sharpness of the melting point would indicate only one aldehyde. The cobalt compound was prepared from the Schiff's base in a hot, dilute sodium hydroxide solution. The brown precipitate which formed was filtered off, washed, and dried in a vacuum at 100° and at 170°. It was inactive.

DI-(2-HYDROXY-3-CHLOROBENZAL)-ETHYLENEDIIMINE

2-Hydroxy-3-chlorobenzaldehyde (synthesized from *o*-chlorophenol by the Duff reaction, m.p.: 52°) and ethylenediamine were condensed in alcohol; m.p.: 143°.

DI-(2-HYDROXY-3-CHLOROBENZAL)-ETHYLENEDIIMINE COBALT

To 2 g. of the Schiff's base dissolved in 20 ml. of alcohol was added the equivalent amount of cobalt acetate in 20 ml. of water. The red-brown material which separated was filtered off, washed with water, and dried in a vacuum at 100°. The material carried 1.15 per cent oxygen reversibly. The oxygen capacity was increased to 1.88 per cent when the material was heated in a vacuum at 150°. The rate at which this compound absorbed oxygen, however, was very low.

A second preparation made in the same way but from hot alcohol had an oxygen-carrying capacity of only 0.5 per cent.

DI-(2-HYDROXY-3-BROMOBENZAL)-ETHYLENEDIIMINE COBALT

The condensation in hot alcohol of 2-hydroxy-3-bromobenzaldehyde (prepared by the Duff reaction, m.p.: 49°) and ethylenediamine yielded a yellow Schiff's base; m.p.: 174°.

DI-(2-HYDROXY-3-BROMOBENZAL)-ETHYLENEDIIMINE COBALT

To 4.25 g. of the Schiff's base and 0.8 g. of sodium hydroxide dissolved in 200 ml. of water was added a hot solution of 2.37 g. of cobalt chloride dissolved in 200 ml. of water containing a few drops of acetic acid. A dark-brown precipitate formed quickly. This was filtered off and dried at 100°. It failed to absorb oxygen when subjected to oxygen at 200 pounds pressure. Heating to 170° in a vacuum failed to activate the material.

Further preparations of the compound were made by grinding the Schiff's base and cobalt acetate together in water and digesting and by bringing the base and cobalt salt together in 70 per cent alcohol, but both procedures led to an inactive product.

2-HYDROXY-5-BROMOBENZALDEHYDE

This aldehyde was prepared by the direct bromination of salicylaldehyde in acetic acid (14); m.p.: 104°–106°; reported (14): 104°–105°.

DI-(2-HYDROXY-5-BROMOBENZAL)-ETHYLENEDIIMINE

The condensation of the aldehyde and diamine was effected in absolute alcohol. The yellow crystals were recrystallized from alcohol; m.p.: 188°–188.5°.

DI-(2-HYDROXY-5-BROMOBENZAL)-ETHYLENEDIIMINE COBALT

To a solution of 4.25 g. of di-(2-hydroxy-5-bromobenzal)-ethylenediimine in 100 ml. of hot alcohol was added 2.49 g. of cobalt acetate dissolved in 40 ml. of hot water. The red-brown precipitate which formed immediately was filtered off and washed with water. On drying at 100° the color of the precipitate changed to a brownish yellow. The material did not gain in weight when subjected to oxygen at 200 pounds pressure.

A second preparation made in absolute alcohol from the potassium salt of the Schiff's base and anhydrous cobalt chloride yielded the dark-yellow material directly. It was inactive toward oxygen.

Neither of these preparations lost weight on further heating to 170° in a vacuum, and neither became active.

DI-(2-HYDROXY-3-CHLORO-5-*tert*-BUTYLBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared by dissolving the Schiff's base, di-(2-hydroxy-3-chloro-5-*tert*-butylbenzal)-ethylenediimine, m.p.: 115° (6), in a hot, 70 per cent alcohol and adding the requisite quantity of cobalt acetate in a hot, aqueous solution. The compound was isolated, dried, and tested in the usual manner and found to be inactive.

DI-(2-HYDROXY-3-*iso*-PROPYL-5-CHLORO-6-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared from the Schiff's base, di-(2-hydroxy-3-*iso*-propyl-5-chloro-6-methylbenzal)-ethylenediimine, m.p.: 171° (6), and cobalt acetate by adding a solution of the base in sodium hydroxide to an aqueous solution of the salt. After digestion the precipitate was filtered, washed, and dried at 100°. It was inactive even after being heated to 175°.

DI-(2-HYDROXY-5-CHLORO-6-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base, di-(2-hydroxy-5-chloro-6-methylbenzal)-ethylenediimine, m.p.: 221° (6), dissolved in alcohol was digested with a hot, aqueous solution of cobalt acetate. The final alcohol concentration was about 40 per cent. The compound was inactive.

DI-(2-HYDROXY-3,5-DIBROMBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared from the Schiff's base di-(2-hydroxy-3,5-dibrombenzal)-ethylenediimine, m.p.: 247° (6). The Schiff's base was

dissolved in *n*-propylalcohol and was added to a hot, aqueous solution of cobalt acetate.

A dark brown compound formed which was filtered, washed, and dried at 100° and 175° in a vacuum. It was inactive toward oxygen.

DI-(2-HYDROXY-3-BROMO-5-*tert*-BUTYLBENZAL)-ETHYLENEDIIMINE COBALT

This compound was prepared from the Schiff's base, di-(2-hydroxy-3-bromo-5-*tert*-butylbenzal)-ethylenediimine, m.p.: 120° (6), dissolved in a dilute solution of the necessary amount of sodium hydroxide by treatment with an aqueous solution of cobalt acetate. A red compound was precipitated which was filtered, dried, and heated in a vacuum to 175° and found to be inactive.

DI-(2-HYDROXY-5-NITROBENZAL)-ETHYLENEDIIMINE

One-tenth mole of ethylenediamine monohydrate was added to a hot, alcohol solution of 0.2 mole of 2-hydroxy-5-nitrobenzaldehyde (see Paper V for preparation and separation from 2-hydroxy-3-nitrobenzaldehyde; m.p. after recrystallization from alcohol: 126°). A fine, yellow precipitate formed immediately which was almost insoluble in the hot alcohol. The precipitate was filtered off, washed generously with water, and dried in air; yield: almost quantitative; m.p.: above 250°. No solvent was found from which the material could be recrystallized. It was soluble in dilute potassium hydroxide.

DI-(2-HYDROXY-5-NITROBENZAL)-ETHYLENEDIIMINE COBALT

The Schiff's base, di-(2-hydroxy-5-nitrobenzal)-ethylenediimine, and cobalt acetate were mixed in the molecular ratio 1:1.5 and ground well together in a mortar. The mixture was then digested at 100° for 15 minutes, during which it turned dark red in color. The precipitate was filtered off, washed with water, and dried in a vacuum at 100°. A second preparation was made by treating a solution of the Schiff's base in a potassium hydroxide solution with an equivalent amount of cobalt acetate dissolved in water. In this case the cobalt compound precipitated immediately, and in nearly quantitative yield. Both preparations were heated to 170° in a vacuum but were inactive toward oxygen.

DI-(2-HYDROXY-3-NITRO-5-METHYLBENZAL)-ETHYLENEDIIMINE

To 28.1 g. of 2-hydroxy-3-nitro-5-methylbenzaldehyde (m.p.: 139°; prepared by nitration of 2-hydroxy-5-methylbenzaldehyde in acetic acid solution (15,16)) dissolved in 1.2 l. of alcohol, was added 6.75 g. of 68.7 per cent ethylenediamine in 300 ml. of alcohol. The orange yellow Schiff's base, which precipitated immediately, was filtered off and dried; m.p.: above 225°.

DI-(2-HYDROXY-3-NITRO-5-METHYLBENZAL)-ETHYLENEDIIMINE COBALT

Ten g. of the Schiff's base was dissolved in 150 ml. of hot water containing 2 g. of sodium hydroxide. To the resulting, blood-red solution was added 10 g. of cobalt chloride and 5 ml. of acetic acid in 50 ml. of warm water. The mixture was digested 15 minutes on a hot plate and the dark red precipitate then filtered off and dried in a vacuum at 100° and at 150°. The material was inactive toward oxygen.

A second preparation was made using *n*-propyl alcohol as solvent and using no sodium hydroxide. The product was also inactive.

2-HYDROXY-3-METHOXY-5-NITROBENZALDEHYDE

Orthovanillin was nitrated in glacial acetic acid at 10° as described by Murakini (15); m.p.: 138°–139°; reported: 138.5°–140° (15).

DI-(2-HYDROXY-3-METHOXY-5-NITROBENZAL)-ETHYLENEDIIMINE

To 40 g. of 2-hydroxy-3-methoxy-5-nitrobenzaldehyde dissolved in 1 l. of alcohol was added 12 g. of ethylenediamine. The yellow precipitate which formed immediately was filtered off and dried; m.p.: above 260° with decomposition.

DI-(2-HYDROXY-3-METHOXY-5-NITROBENZAL)-ETHYLENEDIIMINE COBALT

A fine suspension of 4 g. of the Schiff's base and 2.5 g. of cobalt acetate in 150 ml. of water was boiled for 5 minutes. The red precipitate which formed was filtered off, dried in a vacuum at 100° and at 170°, and subjected to the usual test. It was inactive toward oxygen.

DI-(*o*-HYDROXYACETOPHENO)-ETHYLENEDIIMINE

o-Hydroxyacetophenone and ethylenediamine were condensed in absolute alcohol; m.p.: 195°.

DI-(*o*-HYDROXYACETOPHENO)-ETHYLENEDIIMINE COBALT

The Schiff's base was dissolved in hot, 70 per cent alcohol and a hot, aqueous solution of the necessary amount of cobalt acetate added. The orange-red precipitate which formed immediately was filtered, and washed and dried at 100° in a vacuum. This compound carried about 4 per cent of oxygen reversibly but absorbed the oxygen quite slowly at room temperature even when subjected to oxygen at 200 pounds pressure.

2-HYDROXY-3-NITROACETOPHENONE

o-Hydroxyacetophenone was nitrated directly in glacial acetic acid with concentrated nitric acid. The material was isolated by steam distillation of the diluted reaction mixture. Neutralization of the acids prior to the steam distillation did not improve the poor yield nor did ether extraction of the neutralized reaction mass. The material was obtained as white needles having a barely perceptible tinge of yellow; m.p.: 97°–98°; reported: 98.5°–99.5° (18).

DI-(2-HYDROXY-3-NITROACETOPHENO)-ETHYLENEDIIMINE

2-Hydroxy-3-nitroacetophenone, m.p.: 97°–98°, was condensed with ethylenediamine in boiling alcohol. A quantitative yield of yellow crystals was obtained; m.p.: above 215°.

DI-(2-HYDROXY-3-NITROACETOPHENO)-ETHYLENEDIIMINE COBALT

Because of the insolubility of the Schiff's base in alcohol and water it was necessary to form the cobalt derivative by triturating the base with a solution of the necessary amount of cobalt acetate and warming the suspension for some time. A dark red, insoluble material was obtained which was apparently the compound desired. It did not carry oxygen after drying at 100° or at higher temperatures.

DI-(2-HYDROXY-4-METHYLACETOPHENO)-ETHYLENEDIIMINE

2-Hydroxy-4-methylacetophenone, synthesized from *m*-cresol acetate by the Fries reaction, b.p.: 208°/740 mm., was condensed with ethylenediamine in alcohol and the yellow Schiff's base recrystallized from *n*-propyl alcohol; m.p.: 223°.

DI-(2-HYDROXY-4-METHYLACETOPHENO)-ETHYLENEDIIMINE COBALT

This cobalt compound was prepared by the addition of an aqueous solution of cobalt acetate to a hot solution of the Schiff's base in *n*-propyl alcohol. A brown compound was obtained which was dried in a vacuum at 100° and at 170°. It was inactive toward oxygen.

DI-(2-HYDROXY-5-METHYLACETOPHENO)-ETHYLENEDIIMINE

2-Hydroxy-5-methylacetophenone was prepared from *p*-cresylacetate by the Fries reaction; yield 86 per cent, cry. p.: 45°; b.p.: 209°/740 mm. The condensation of this acetophenone and ethylenediamine was effected in alcohol, the reaction being rather slow; yellow crystals, m.p.: 198°.

DI-(2-HYDROXY-5-METHYLACETOPHENO)-ETHYLENEDIIMINE COBALT

This compound was prepared by the addition of a hot, aqueous solution of 2.5 g. of cobalt acetate to a solution of 7.0 g. of Schiff's base in alcohol. The red compound which formed immediately was filtered off and dried in a vacuum at 100°. When heated in a vacuum at 130° and 175° it experienced a very small loss in weight. When subjected to oxygen at 200 pounds pressure it gained 1 per cent in weight although there was no change in color. The oxygen was expelled when the material was heated to 100°. The capacity did not improve on repeated cycling. The compound was not appreciably hygroscopic since the weight remained constant upon exposure to the atmosphere for several minutes.

DI-(RESACETOPHENO)-ETHYLENEDIIMINE

To a solution of 11.2 g. of resacetophenone in 200 ml. of alcohol was added a cold solution of 3.61 g. of ethylenediamine in 200 ml. of alcohol. On standing a pinkish yellow precipitate formed which was filtered off and

dried. This material was insoluble in water, benzene, petroleum ether, dioxane, cyclohexane, toluene, and chloroform.

DI-(RESACETOPHENO)-ETHYLENEDIIMINE COBALT

To a solution of 5.3 g. of the Schiff's base in 150 ml. of water containing 1.5 g. of sodium hydroxide was added a solution of 9 g. of cobalt acetate in 25 ml. of water. A brownish-red precipitate formed immediately which was filtered off and dried in a vacuum at 100°. This material gained 0.3 per cent in weight in oxygen at 200 pounds pressure. After being heated to 170° in a vacuum it failed to absorb any oxygen.

A second preparation made in hot water was lighter brown in color and absorbed only 0.1 per cent oxygen after vacuum drying at 100° but again no oxygen after heating to 170° in a vacuum.

DI-(2-HYDROXYPROPIOPHENO)-ETHYLENEDIIMINE

Propiophenone, b.p.: 110°/6 mm., was condensed with ethylenediamine in alcohol. The yellow crystals obtained were recrystallized from alcohol, m.p.: 118°–118.5°.

DI-(2-HYDROXYPROPIOPHENO)-ETHYLENEDIIMINE COBALT

An attempt to prepare this cobalt compound by adding a hot aqueous solution of cobalt acetate to a hot alcoholic solution of the Schiff's base yielded only a brown oily material. Several other methods also failed to yield a solid cobalt compound.

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STUDIES ON OXYGEN-CARRYING COBALT COMPOUNDS

IX. Analogues of Disalicylalethylenediimine Cobalt with Diamines Other Than Ethylenediamine

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The intriguing property of disalicylalethylenediimine cobalt of reversibly absorbing and releasing oxygen lead naturally to an investigation of the compounds in which the ethylenediamine was replaced by other diamines. It was expected that a minor change in the composition of the compound, such as obtained by passing from ethylenediamine to propylenediamine, would modify only slightly the physical properties of the compound. Such minor modifications of the physical characteristics would be of importance from an engineering viewpoint in adapting the materials to the manufacture of oxygen; for example, a temperature of maximum rate of oxygenation of 30° would be distinctly more advantageous than one of 15° since it would permit the use of cooling water during the oxygenation phase of the cycle rather than more complicated mechanical refrigeration needed for the lower temperature.

The results were quite at variance with this simple concept, however. Of some sixteen diamines other than ethylenediamine, not one yielded a cobalt derivative which exhibited any tendency to absorb oxygen. These included diamines in which the amino groups were located on adjacent carbon atoms but in which one or more hydrogen atoms were replaced by methyl groups, and other amines in which the ethylene group was either absent (hydrazine) or replaced by $-CH_2-$ chains of various lengths.

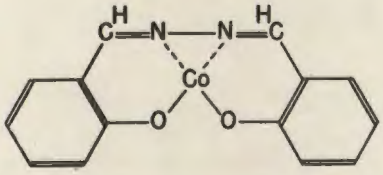
Crystalline Schiff's bases were obtained from salicylaldehyde and the majority of the diamines. These bases were invariably bright yellow in color and possessed sharp melting points.

The cobalt derivatives were generally prepared by several methods since it was shown in the case of the parent oxygen-carrying compound, disalicylalethylenediimine cobalt, that the capacity to absorb oxygen varied with the method of preparation. The preferred method was that of adding a solution of the Schiff's base in alcohol to an aqueous solution of cobalt acetate. Occasionally alkali was necessary to assist in the solution of the Schiff's bases but in those cases care was exercised to avoid excess alkali, known in the case of the parent compound, to lead to an inactive, isomeric form of the compound. Direct mixing of the diamine, salicylaldehyde and cobalt salt was also used frequently. The method of first forming a pyridine addition compound and subsequently driving off the pyridine at high temperature was also used in a number of instances.

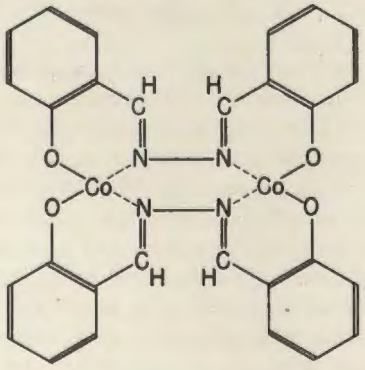
As shown in the studies of the 3-nitro and 3-methoxy derivatives of the parent compound it is sometimes necessary to expel water from the materials before they become capable of absorbing oxygen. The compounds prepared in the present investigation were therefore heated in a vacuum at temperatures as high as 170° before being abandoned as nonactive.

The ability to absorb oxygen was determined by placing the materials in oxygen at 200 pounds per square inch pressure in the apparatus described in Paper XIII of this series, and noting any increase in weight.

The stereochemical implications of the failure of these compounds to absorb oxygen escape us for the present. It is unlikely in the case of the compound derived from hydrazine that the structure is



since a three membered ring would be present. More likely is the structure:



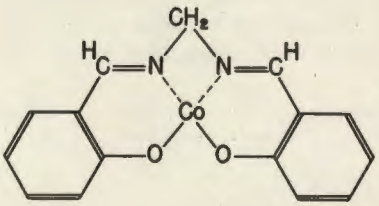
The question of the molecular weights and structure of similar compounds has been treated by Pfeiffer (1).

Of the polymethylenediamines, $H_2N\text{-}(CH_2)_n\text{-}NH_2$, an oxygen carrier is obtained only when n equals two, indicating that a five-membered ring must be present in the compound. In the case of the methyl substituted ethylenediamines such as propylenediamine, 2,3-butylenediamine, and tetramethylethylenediamine, a five-membered ring is present, but the compounds are inactive toward oxygen. No adequate explanation is available for this difference in behavior.

The preparation of the hydrazine compound, disalicylazine cobalt, presented no difficulty. The Schiff's base, disalicylazine, had been previously reported. The cobalt compound prepared by two methods was

found to be red brown in color, insoluble, and inactive toward oxygen. This compound was not characterized as well as might be desirable since our interest was simply in its behavior toward oxygen. This compound presents an interesting problem in stereochemistry which might well be the subject of some future investigation.

Methylenediamine, $H_2N\text{-}CH_2\text{-}NH_2$, would be expected to yield a Schiff's base with salicylaldehyde which would subsequently yield a cobalt compound containing a four-membered ring:



Four-membered rings are well-known in chelate ring chemistry and it is likely that the structure of the cobalt-salicylaldehyde derivative is that represented by the above formula. The instability of methylenediamine as the free base made it necessary to prepare the Schiff's base from the hydrochloride at low temperature. The cobalt derivative was prepared without difficulty.

Propylenediamine is now commercially available; indeed, the compound disalicylalpropylenediimine is marketed as an 80 per cent solution in xylene by the du Pont Company as a deactivator to remove from gasoline the metals which promote gum formation (2). Disalicylalpropylenediimine was, however, obtained as a liquid in contrast to disalicylalethylenediimine which is a beautiful, crystalline solid, melting at 127°. The propylenediamine was carefully fractionated and an extended purification of disalicylalpropylenediimine carried out. The final product was gummy in character, and no sharp melting point was obtained. The cobalt derivative of this material was prepared in water and in water-alcohol mixtures, but none of the products absorbed oxygen.

It was thought that the presence of an asymmetric carbon atom in the propylenediamine molecule accounted for the failure of the condensation product with salicylaldehyde to crystallize, it being known that racemic mixtures do not in general crystallize easily. For this reason it was thought that the optically active forms of propylenediamine might yield cobalt compounds which would function as oxygen carriers. Accordingly, propylenediamine was resolved by fractional crystallization of the acid tartrate. The Schiff's base, prepared directly from the acid tartrate was obtained as an oil. The cobalt derivative was found to be inactive toward oxygen.

The synthesis of disalicylalpropylenediimine cobalt through a pyridine addition product was also carried out. A crystalline pyridine addition compound was obtained, which, after drying, was heated in a vacuum at

170° to expel the pyridine. The residual material was found not to carry oxygen.

Disalicylaltrimethylenediimine was synthesized and found to be a low-melting, yellow crystalline solid. Its cobalt derivative was prepared. Analysis indicated that the material obtained was not the compound expected, but since the material obtained did not carry oxygen, it was not investigated further.

The highly symmetrically substituted ethylenediamine, tetramethylethylenediamine, $(CH_3)_2C(NH_2)C(NH_2)(CH_3)_2$, was obtained from the Commercial Solvents Corporation. Its identity and purity were checked and its condensation with salicylaldehyde effected in absolute alcohol. The cobalt derivative of the condensation product was prepared by several methods and numerous attempts were made to activate the compound by various heat treatments. None of the preparations functioned as an oxygen carrier.

The presence of a nitro or a methoxy group in the 3- position of salicylaldehyde has been shown to have a beneficial effect on the properties of the parent oxygen-carrying compound. It seemed possible that the presence of these groups might render the tetramethylethylenediamine compound active, and accordingly the cobalt derivatives of the condensation products of 3-nitro and 3-methoxysalicylaldehyde and tetramethylethylenediamine were prepared and subjected to the usual tests. All of the preparations were found to be inactive, thus further confirming the general conclusion that variations in the ethylenediamine portion of the parent compound always leads to a material which is not an oxygen carrier.

2,3-Butylenediamine was prepared by converting dimethylgyoxime to its diacetate by treatment with acetic anhydride, and reducing the diacetate with metallic sodium. The amine was then condensed with salicylaldehyde and the product converted into the cobalt salt. The latter was a bright-red, crystalline material which did not function as an oxygen-carrier.

The Schiff's base of hexamethylenediamine and salicylaldehyde was just as easily obtained. Several preparations of the cobalt compound were made using different procedures, but none of the products carried oxygen.

Disalicylalnonamethylenediimine, synthesized from nonamethylenediamine and salicylaldehyde, was obtained as a yellow crystalline material. Its cobalt derivative was obtained as a tar which could not be induced to crystallize. The dried and ground material was found not to carry oxygen.

Disalicylaldecamethylenediimine was synthesized and found to be a crystalline yellow solid. Its cobalt derivative was obtained as a tar which on desiccation and grinding gave a powder which was inactive toward oxygen. The materials were tested for oxygen absorption by treatment with oxygen at 100 pounds pressure as described in Paper XIII.

The aromatic base, *o*-phenylenediamine, also yields a Schiff's base with two molecules of salicylaldehyde, although it is orange in color in

contrast to the invariable yellow of the bases of aliphatic diamines. The cobalt derivative, however, did not absorb oxygen.

Several more complex amines, available commercially, were also studied; the cobalt compounds prepared were inactive toward oxygen without exception. Pentaerythrityltetramine, $C(CH_2NH_2)_4$, for example, condensed readily with salicylaldehyde but yielded an inactive cobalt derivative.

Diethylenetriamine, $H_2N\text{-}C_2H_4\text{-}NH\text{-}C_2H_4\text{-}NH_2$, and triethylenetetramine, $H_2N\text{-}C_2H_4\text{-}NH\text{-}C_2H_4\text{-}NH\text{-}C_2H_4\text{-}NH_2$, were condensed with salicylaldehyde and also with 2-hydroxy-3-nitro- and 2-hydroxy-5-nitrobenzaldehyde. The Schiff's bases derived from these aldehydes were viscous oils which could not be induced to crystallize. The cobalt derivatives were prepared from the oily bases but were found to be inactive. Because of the high melting points of 2-hydroxy-3-nitrobenzaldehyde and of 2-hydroxy-5-nitrobenzaldehyde, it was expected that a crystalline Schiff's base might be obtained, and this proved to be the case. The cobalt derivatives of the Schiff's bases were also more crystalline in character but were again all inactive toward oxygen. Care was taken in the case of 2-hydroxy-3-nitrobenzaldehyde to obtain a pure preparation of the aldehyde, that is, one melting about 109°, since, as was shown in Paper V of this series, 2-hydroxy-3-nitrobenzaldehyde having a low melting point leads to a cobalt-ethylenediamine compound inactive toward oxygen.

EXPERIMENTAL WORK

DISALICYLAZINE COBALT

Two equivalents of salicylaldehyde and one of hydrazine sulfate were mixed in warm alcohol. The condensation proceeded immediately forming a yellow, crystalline precipitate which was filtered off, twice recrystallized from 50 per cent alcohol, and dried. M.p.: 216°; m.p. reported (3): 213°–214°; the thermometer used was calibrated at 212° with anthracene, and our value is probably better.

A solution of 7.8 g. of disalicylazine dissolved in alcohol was added with vigorous stirring to a solution of 13 g. of cobalt acetate dissolved in warm water. A red-brown precipitate formed which was filtered off and dried in a vacuum at 100°. It did not gain in weight or change color when subjected to oxygen at 200 pounds pressure. When heated at 150° in a vacuum for some time it lost weight to some extent but remained inactive toward oxygen.

A second preparation of this cobalt compound made by simultaneous mixing of hydrazine sulfate, salicylaldehyde, and cobalt acetate was similar in appearance and also inactive toward oxygen.

DISALICYLALMETHYLENEDIIMINE COBALT

A sample of methylenediamine hydrochloride was obtained from E. I. du Pont de Nemours & Co. Preliminary attempts to prepare the Schiff's base by adding sodium hydroxide to a solution of methylenediamine

hydrochloride and salicylaldehyde in absolute alcohol yielded an oil from which only a very small quantity of the base could be isolated. A reasonable yield was finally obtained by carrying out the neutralization in the cold and minimizing the heating necessary to effect the condensation. To a solution of 16 g. of sodium hydroxide in 25 ml. of water, cooled in an ice bath, was added a solution of 16.5 g. of methylenediamine hydrochloride in 25 ml. of water cooled at 0°. After 30 seconds this mixture was added to a warm solution of 48 g. of salicylaldehyde in 250 ml. of alcohol. The mixture was stirred vigorously and then allowed to cool to room temperature. On chilling further in an ice bath yellow crystals were deposited which were filtered off, washed with water, and dried. Yield: fair; m.p.: 132°. Hot solutions of this Schiff's base in alcohol had a strong odor of salicylaldehyde, indicating that it is easily hydrolyzed.

Two preparations of the cobalt derivative were made. In the first, the Schiff's base was dissolved in water containing sufficient sodium hydroxide to make the sodium salt of the base. To this was added a solution containing the equivalent amount of cobalt acetate well buffered with acetic acid and sodium acetate. The mixture was digested on a steam bath a few minutes, cooled, and filtered, and the yellow product washed with water and dried in air at 100°. The dried material was then heated in a vacuum at 170° for 2 hours and subjected to oxygen at 200 pounds pressure. It did not gain in weight or change in color. A second preparation was made using an alcohol solution of the Schiff's base with no sodium hydroxide, the volumes of the alcohol solution and the aqueous solution of cobalt acetate being so adjusted that the final reaction mixture was about 50 per cent alcohol. The product was identical in appearance and also inactive. It seemed possible that since the Schiff's base was so easily hydrolyzed that the material obtained might have been cobalt salicylaldehyde. The compound contained nitrogen, however, and an analysis for cobalt indicated that the material was undoubtedly disalicylalmethylenediimine cobalt. Found: 18.8, 18.7 per cent cobalt (evaporation with nitric and sulfuric acids and ignition at 500° to cobalt sulfate); calculated for $C_{15}H_{12}O_2N_2Co$: 18.9 per cent cobalt.

Unfortunately, however, this compound did not gain in weight or change in color on being subjected to oxygen at 200 pounds pressure.

DISALICYLALPROPYLENEDIIMINE COBALT

Commercial propylenediamine (Carbide and Carbon Chemicals Corporation) was carefully fractionated; b.p.: 115°–116°/745 mm.; reported 119°/760 mm. A sample of the distilled propylenediamine weighed in a glass stoppered bottle and opened under water was titrated with standard hydrochloric acid to the methyl red end-point. Found: 81.5 per cent propylenediamine; calculated for propylenediamine monohydrate: 80.5 per cent.

The literature contains relatively little on the Schiff's base, disalicylalpropylenediimine. Pfeiffer (4) describes the copper derivative of the compound but does not discuss the compound itself.

To 244 g. of salicylaldehyde was added 90 g. of propylenediamine slowly with vigorous stirring and cooling under running tap water. The product was a heavy, clear, yellow oil. This oil was beaten together with 300 ml. of hot water, forming a fine emulsion. After cooling and standing, the water layer was separated, carrying away any water-soluble impurity. This process was repeated with hot alcohol, in which the compound is fairly soluble. On cooling to 0°, the disalicylalpropylenediimine separated as a very viscous layer from which the alcohol was poured off. The oily layer was then heated to 110° for 30 minutes to drive off any dissolved water or alcohol. The final product was a viscous liquid. Upon standing for several days at room temperature, feathery crystals began to form. On standing for another week, the condensation product changed to a solid mass, but material having a sharp melting point was not obtained.

The cobalt compound, disalicylalpropylenediimine cobalt, was prepared by several methods. In 1.5 l. of hot water containing 15.9 g. of sodium hydroxide and 5 g. of sodium acetate, $NaC_2H_3O_2 \cdot 3H_2O$, was dissolved 56.6 g. (0.2 mole) of disalicylalpropylenediimine. To this solution was added with vigorous agitation a solution of 58.6 g. (0.2 mole) of cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$, in 100 ml. of hot water. The solution became yellow orange in color, and a red-brown precipitate formed on shaking and cooling to 40°. The material was centrifuged and washed with 1 l. of distilled water. The material was dried for 3 hours at 100° in a vacuum. Yield: 55 g., 81 per cent; oxygen absorption at 200 pounds oxygen pressure, room temperature: 0.05 per cent. The material was not hygroscopic.

This preparation was repeated making certain that no free alkali was present which was known to lead to an inactive red isomer in the case of the corresponding ethylenediamine compound, but again the material failed to absorb oxygen.

Another preparation was carried out in which an alcohol solution of disalicylalpropylenediimine was added to an aqueous solution of cobalt acetate, the final mixture being about 80 per cent alcohol. The addition of water did not precipitate the cobalt compound. In another preparation where the final alcohol concentration was about 50 per cent, a red-brown precipitate formed immediately. This was centrifuged from the solution washed with a large volume of water, and dried in a vacuum at 100°. The material was inactive toward oxygen.

Disalicylalpropylenediimine cobalt was also prepared through its pyridine addition compound. To 6.28 g. of propylenediamine, 22 g. of cobaltous chloride, $CoCl_2 \cdot 6H_2O$, and 36.5 ml. of pyridine dissolved in hot water was added 20 ml. of salicylaldehyde. The vessel was evacuated immediately by means of a suction pump, and evacuation was continued for 30 minutes, and at the same time the mixture was kept warm on a hot plate, the mass being shaken frequently. A dark-red precipitate formed which was filtered off after cooling, washed with cold water, pressed between filter papers at about 1,600 pounds pressure to expel liquid, and dried for several hours, in a vacuum at 170°. The material did not gain in weight when subjected to oxygen at 200 pounds pressure.

The Schiff's base of *l*-propylenediamine was prepared directly from the *l*-propylenediamine bitartrate by dissolving the latter in excess of potassium hydroxide and adding two equivalents of salicylaldehyde. Reaction occurred immediately, forming a small amount of crystalline material which was isolated and found to be inactive. The filtrate was carefully neutralized with hydrochloric acid causing the separation of an oil which on standing in contact with the mother liquor gave a further amount of a yellow crystalline material, which was found to be inactive.

The residual, red oil was washed by shaking it with water, dried, and found to be active; $[\alpha]^{29} = -192.2°$. It was apparently disalicylal-*l*-propylenediimine and was used in the preparation of a cobalt compound.

Several attempts to prepare the cobalt derivative of disalicylal-*l*-propylenediimine by the addition of an alcohol solution of the active Schiff's base to an aqueous solution of a cobalt salt led to oily products of uncertain composition. A red precipitate was only obtained when the volumes of the reacting solutions were adjusted so that the final reaction mixture was less than 50 per cent alcohol. The material finally secured was similar in appearance to the oxygen carrier disalicylalethylenediimine cobalt, but was inactive toward oxygen. Even after being heated in a vacuum at 170° for 3 hours, a process which activates certain materials, for example di-(2-hydroxy-3-methoxybenzal)-ethylenediimine cobalt, it did not gain in weight when subjected to oxygen at 200 pounds pressure.

DISALICYLALTRIMETHYLENEDIIMINE COBALT

Trimethylenediamine obtained from Sharples Chemicals, Inc. was fractionated; b.p.: 134°–137°/735 mm.; reported (7): 135°–136°/738 mm. Titration with standard acid indicated that the material used contained 93 per cent of the base.

On the slow addition of 0.5 mole of trimethylenediamine to 1 mole of salicylaldehyde reaction occurred as evidenced by the generation of heat and the formation of steam. The liquid condensation product was induced to crystallize on cooling in ice. It was then dissolved in alcohol, and the concentration was so adjusted that the condensation product remained in solution until the solution was cooled below the melting point of the crystallized condensation product. To obviate the low yield obtained by this technique the major part of the Schiff's base was thrown out of the alcohol solution by the addition of ice. This slow dilution coupled with vigorous agitation gave an excellent product. The disalicylaltrimethylenediimine was finally filtered cold and dried in a desiccator which was stored in a refrigerator. The material could not be distilled under vacuum: m.p.: 50°–51°.

The cobalt derivative of this Schiff's base was prepared by the addition of an alcoholic solution of it to an aqueous solution of cobalt acetate. A tan material precipitated which was filtered, washed with water, and dried. It did not gain in weight when subjected to oxygen at 200 pounds pressure. The product was analyzed for nitrogen by a modified Kjeldahl procedure in which the digestion was begun with dilute sulfuric acid and

for cobalt by the ferricyanide titration method. Found: 5.92, 6.09 per cent nitrogen, 19.05, 19.15 per cent cobalt; calculated for $C_{17}H_{16}O_2N_2Co$: 8.25 per cent nitrogen, 17.4 per cent cobalt.

These analyses give a cobalt to nitrogen ratio of 3:2 rather than the desired 1:2, showing that the material prepared was not disalicylaltrimethylenediimine cobalt. Since the material did not carry oxygen no further attention was paid to it.

DISALICYLALTETRAMETHYLETHYLENEDIIMINE COBALT

Tetramethylethylenediamine was obtained from the Commercial Solvents Corporation as the free base in colorless, deliquiescent crystals, melting gradually from 86°–93° (reported by Pirinskimi (8): 97°–99°). Titration of a sample with standard hydrochloric acid indicated that the material contained 82.5 per cent tetramethylethylenediamine.

The Schiff's base disalicylaltetramethylethylenediimine formed readily on mixing alcohol solutions of two equivalents of salicylaldehyde and one equivalent of tetramethylethylenediamine. It was necessary to add a small amount of water to induce crystallization. The light yellow crystals were recrystallized from alcohol. Yield: 13.4 g., 41.6 per cent; m.p.: 114.5°. Found: 8.30, 8.35, 8.39 per cent nitrogen by the modified Kjeldahl method; calculated for $C_{20}H_{24}O_2N_2$: 8.64 per cent nitrogen.

Several preparations of the cobalt derivative of this Schiff's base were made in an effort to find conditions which would lead to a compound active toward oxygen. In one preparation 58.6 ml. of 1.087 *N* sodium hydroxide was added to 10.3 g. of disalicylaltetramethylenediimine and heated to 60° with 25 ml. of alcohol. A deep-red solution resulted which darkened as the heat was increased. One equivalent of cobalt chloride, 7.56 g. of $CoCl_2 \cdot 6H_2O$, in solution with 15 ml. of water and 10 drops of acetic acid, was added to this solution. After shaking, large lumps of light-brown material precipitated. This precipitate was washed with water and dried in air. Yield: 7.8 g., 62.7 per cent. On heating to 170° in vacuum the entire material became darker, and the edges showed evidences of charring. There was a weight loss of 17.1 per cent. It did not gain weight when subjected to oxygen at 200 pounds pressure.

In the next preparation the acetic acid was omitted, but the product was the same and again inactive. In a third preparation the effect of *p*H was studied more closely. A solution of the sodium salt of the Schiff's base in 25 per cent alcohol gave a reading of 11.6 using a glass electrode. This was reduced to 9.5 by the addition of acetic acid but it became necessary to add more alcohol to keep the material in solution. On the addition of an acid solution of cobalt chloride, *p*H 1.5, a light-brown precipitate formed which was filtered and washed with water. The black filtrate was nearly neutral, *p*H of 7.5. The brown product was definitely crystalline. After drying at 155° in a vacuum it failed to gain weight in oxygen at 180 pounds pressure.

In another preparation no alcohol or acid was used, and the sodium hydroxide was added in small amounts during digestion. Complete

solution did not occur owing to formation of some cobalt hydroxide. The final product was light red in color and was heated at several temperatures from 100° to 170° but was found to be inactive.

A further preparation was carried out by the pyridine method. To a mixture of 5 g. of tetramethylethylenediamine, 8.66 g. of salicylaldehyde, 8.41 g. (three equivalents) of pyridine, and 80 ml. of water, was added a solution of 8.60 g. of cobalt chloride in 20 ml. of water. The mixture was stirred vigorously and digested on a steam bath for 1 hour. A dark-red precipitate was obtained, which was filtered off, washed with water, and dried in air at 100°. Yield: 13.2 g., 96 per cent. The odor of pyridine was still apparent. On being heated at 140° in a vacuum this material lost 3 per cent in weight, and lost an additional 2.98 per cent at 170°. After each treatment it did not gain weight in oxygen at 200 pounds pressure.

All of the preparations mentioned above were analyzed for cobalt by decomposition with sulfuric acid followed by the ferricyanide titration. The values found for cobalt were within 0.5 per cent of the theoretical value of 15.47, indicating that the materials obtained were undoubtedly the cobalt compound expected.

DI-(2-HYDROXY-3-METHOXYBENZAL)-TETRAMETHYLETHYLENEDIIMINE COBALT

In this preparation 5 g. of tetramethylethylenediamine and 11 g. of 2-hydroxy-3-methoxybenzaldehyde were mixed in 30 ml. of alcohol. On shaking the mixture reaction suddenly occurred, a nearly solid mass being formed. The material was filtered and washed with a little water. Yield after air drying: 11 g., 70 per cent; m.p.: 182°. Loss on drying in air at 100°: 3.52 per cent.

To 3.78 g. of cobalt chloride, $CoCl_2 \cdot 6H_2O$, in 40 ml. of water was added 5.92 g. of di-(2-hydroxy-3-methoxybenzal)-tetramethylethylenediimine. As this mixture digested, one equivalent of sodium hydroxide as a 1 *N* solution was added slowly. A blood-red precipitate formed which was filtered off and washed with water. Yield after air drying: 7.7 g.; theoretical yield: 7.8 g. Loss on air drying at 105°: 7.16 per cent. When heated in a vacuum at 170° the sample decomposed. A second sample, carefully heated at 140°–155° in a vacuum, became slightly lighter in color and was cooled in a vacuum. Under oxygen at 200 pounds pressure, the original color completely returned, and the sample gained 0.38 per cent in weight.

A second preparation of the cobalt compound was carried out using a 50 per cent alcohol reaction medium, two equivalent of sodium hydroxide, and a temperature of 60° at the time of adding the cobalt chloride. The product was light brown in color, the yield was very low, and the material after vacuum drying at 150° did not gain weight in oxygen at 200 pounds pressure.

DI-(2-HYDROXY-3-NITROBENZAL)-TETRAMETHYLETHYLENEDIIMINE COBALT

2-Hydroxy-3-nitrobenzaldehyde, m.p.: 93°–95°, was dissolved in alcohol and treated with an alcoholic solution of the diamine. The result-

ing, bright-yellow Schiff's base was recrystallized from alcohol; m.p.: 181° with slight decomposition.

A quantity of 3 g. of di-(2-hydroxy-3-nitrobenzal)-tetramethylethylenediimine was dissolved in 13.35 ml. of 1.087 *N* sodium hydroxide containing 0.25 g. of sodium acetate, yielding a red solution upon slight heating. To this was added 1.73 g. of cobalt chloride dissolved in 35 ml. of boiling water and 0.3 ml. of acetic acid. The pasty reaction mass which resulted was filtered, washed with water, and dried at 100°. Yield: 3.20 g., 94 per cent. Samples of the material heated in vacuum at 150°, 170°, and 200° lost 4 to 6 per cent in weight but the products did not absorb oxygen on exposure to oxygen at 200 pounds pressure.

DISALICYLAL-2,3-BUTYLENEDIIMINE COBALT

2,3-Butylenediamine was prepared from the diacetate of dimethylglyoxime by the method of Balthis and Bailar (9). Although this method was found rather unsatisfactory a sufficient amount of the diamine was obtained to prepare a cobalt derivative.

A few crystals of cobalt acetate were dissolved in approximately 12 ml. water. Approximately 5 drops of the 2,3-butylenediamine was added. This solution was mixed with a solution of 5 drops of salicylaldehyde in absolute alcohol. A brilliant orange crystalline precipitate formed which was filtered off, washed with water, and dried in a vacuum at 100°. The product did not carry oxygen.

DISALICYLALHEXAMETHYLENEDIIMINE COBALT

Hexamethylenediamine was obtained from E. I. du Pont de Nemours & Co. as a white crystalline solid; m.p.: 31°–31.5°, reported by Coloniny (10): 30°. The Schiff's base was prepared in the usual manner by mixing one equivalent of the diamine with two equivalents of salicylaldehyde in warm absolute alcohol. On cooling, beautiful, bright crystals of disalicylalhexamethylenediimine, formed; m.p.: 68°–68.5° (from alcohol), 68.5° (from light petroleum ether). Found: 8.52, 8.21 per cent nitrogen, by the modified Kjeldahl method; calculated for $C_{20}H_{24}O_2N_2$: 8.65 per cent nitrogen.

The cobalt derivative was prepared by the addition of an alcohol solution of the Schiff's base to an aqueous solution of cobalt acetate, the quantities used being 9.72 g. of disalicylalhexamethylenediimine in 150 ml. of alcohol and 7.47 g. of cobalt acetate in 100 ml. of water. A black tar formed immediately, but within a few minutes this changed to a red crystalline mass which was filtered off and dried at 100° in a vacuum. This material did not gain weight when subjected to oxygen at 200 pounds pressure. Found: 14.20; 14.27 per cent cobalt; calculated for $C_{20}H_{22}O_2N_2Co$: 15.49 per cent cobalt.

In another preparation an aqueous solution of the sodium salt was prepared by treating a definite amount of sodium hydroxide with an

excess of disalicylalhexamethylenediimine, filtering off the excess, and then adding this solution to an aqueous solution of the required amount of cobalt chloride. The product was yellow green in color and inactive toward oxygen. Found: 15.95, 16.10 per cent cobalt; Calculated for $C_{20}H_{22}O_2N_2Co$: 15.49 per cent cobalt.

The direct simultaneous mixing of an alcohol solution of hexamethylenediamine, an aqueous solution of cobalt acetate, and salicylaldehyde all in the exact amounts required, with subsequent digestion at 100°, gave a red product which was also found to be inactive. Found: 14.50, 14.27, and 14.27 per cent cobalt; calculated for $C_{20}H_{22}O_2N_2Co$: 15.49 per cent cobalt.

DISALICYLALNONAMETHYLENEDIIMINE COBALT

Nonamethylenediamine was obtained from E. I. du Pont de Nemours & Company as a white crystalline solid; m.p.: 35°–38°; reported by Coloniny (11): 37°–37.5°.

The condensation of nonamethylenediamine with two molecules of salicylaldehyde was easily effected in hot, absolute alcohol. The yellow crystalline Schiff's base was recrystallized from alcohol; m.p.: 54.5°–55°. It was also recrystallized from petroleum ether; m.p.: 55°.

The cobalt derivative was prepared by dissolving the Schiff's base in alcohol and adding the warm alcohol solution to a warm aqueous solution of cobalt acetate. Some tan precipitate formed but almost the entire amount of material was obtained as a brown tar. After drying, this product could be crushed to a powder and further dried. The powder did not absorb oxygen when subjected to oxygen at 200 pounds pressure.

DISALICYLALDECAMETHYLENEDIIMINE COBALT

Decamethylenediamine was obtained from E. I. du Pont de Nemours & Company as a white, crystalline solid; m.p.: 61.5°; reported by Phooken and Krafft (12): 61.5°; by Braun (13): 60°. A weighed sample of this material was dissolved in a hot, 25 per cent alcohol solution and titrated with standard acid using methyl red as indicator; the material was found to be 99 per cent decamethylenediamine.

The condensation of salicylaldehyde and decamethylenediamine proceeded immediately on mixing in absolute alcohol. On cooling in ice a beautiful, brilliant yellow, crystalline precipitate formed. This was filtered off, washed with water, and dried. M.p. after recrystallization from alcohol: 65°. Found: 6.64, 7.05, 7.10, 7.00, 6.91, 6.77 per cent nitrogen by the modified Kjeldahl method; calculated for $C_{24}H_{32}O_2N_2$: 7.37 per cent nitrogen; for the monohydrate $C_{24}H_{32}O_2N_2 \cdot H_2O$: 7.00 per cent nitrogen.

Disalicylaldecamethylenediimine was found to be very insoluble in dilute sodium hydroxide making it necessary to use alcohol in preparing the cobalt derivative. Disalicylaldecamethylenediimine cobalt was prepared by dissolving 3.80 g. of disalicylaldecamethylenediimine and 0.4 g.

of sodium hydroxide in absolute alcohol. An aqueous solution of 2.4 g. of cobalt chloride, $CoCl_2 \cdot 6H_2O$, was added to the above solution. Immediately a small amount of fine, tan precipitate formed and with further stirring, a tar also formed which collected on the stirring rod. The tar was dried in a vacuum at 100°; it solidified and was brown in color. This material did not gain in weight on exposure to oxygen at 200 pounds pressure.

It was thought that possibly a crystalline cobalt derivative of disalicylaldecamethylenediimine could be obtained if the preparation were carried out in a very dilute solution. This was tried and a flocculent blue precipitate was obtained. Its appearance was much the same as that of nickel dimethylglyoxime except that it was blue in color. Upon filtering the mixture, so little material was collected that it was discarded and no further work was done along this line.

It has been shown that disalicylalethylenediimine cobalt can be prepared by warming an aqueous paste of an intimate mixture of disalicylalethylenediimine and cobalt acetate together. This method offered a possible way in which to prepare the cobalt derivative of disalicylaldecamethylenediimine. Disalicylaldecamethylenediimine and cobalt acetate were ground together until an intimate mixture was obtained. Water was added and a paste formed. On warming, a brown tar was formed which was dried, ground, and found to be inactive toward oxygen. No practical or successful method of preparing disalicylaldecamethylenediimine cobalt was found.

DISALICYLAL-*o*-PHENYLENEDIIMINE COBALT

A quantity of 12.2 g. (0.1 mole) of salicylaldehyde was dissolved in 25 ml. of 95 per cent ethyl alcohol, and to it was added 5.14 g. of *o*-phenylenediamine (0.05 mole) dissolved in 100 ml. of 95 per cent ethyl alcohol warmed to 50°. The reaction proceeded more slowly than in the case of the corresponding condensation of ethylenediamine with salicylaldehyde. The solution turned yellow immediately, an orange precipitate began to form in about 30 seconds, and the reaction was complete in a few minutes. The product was filtered off, washed with alcohol, and dried at 110°; m.p.: 155°–158°. The material was found to be insoluble in ether, petroleum ether, and carbon tetrachloride. A mixture of benzene and petroleum ether was found to be the most suitable solvent for recrystallization; a mixture of the material and petroleum ether was heated to the boiling point, and benzene was added until all of the orange material had dissolved. The solution was filtered while hot and allowed to stand over night, after which the product was filtered off, washed with petroleum ether, and dried. Three such recrystallizations gave a product melting at 163.5°. A fourth recrystallization from benzene and petroleum ether specially purified for optical rotation work gave a pure product, melting sharply at 165.5°–166°; m.p. reported (14): 166°.

A solution was prepared of 6 g. of disalicylal-*o*-phenylenediimine in 150 ml. of warm water containing 1.5 g. of sodium hydroxide and 1 g. of

sodium acetate. This solution of the sodium salt was added with stirring to a solution containing 4.56 g. of cobalt chloride in 25 ml. of water at 60°. A precipitate formed immediately which was at first an olive green color, but which turned brown in a few minutes. This precipitate was centrifuged and washed with cold water. Approximately one-half of the brown precipitate was dried at 100° under a vacuum for 4 hours. The remaining portion was dried at 170° under a vacuum for 3 hours.

The portion of material which was dried at 100° was powdered in a mortar and weighed samples placed in oxygen at 200 pounds pressure. An increase in weight amounting to 0.1 per cent, but no change in color was observed. Although the material was not noticeably hygroscopic, it is doubtful if the slight increase in weight was due to absorption of oxygen. Upon further drying at 100° this brown compound lost weight and turned a dark red color identical in appearance with the portion of the same preparation originally dried at 170°.

The material dried at 170° became dark red. It was powdered in a mortar and dried to constant weight at this temperature. This material also did not gain weight on exposure to oxygen at high pressure.

A second preparation of this cobalt compound, made using a 50 per cent alcohol reaction medium, was identical in appearance and also inactive.

PENTAERYTHRITYLTETRAMINE AND SALICYLALDEHYDE

Pentaerythrityltetramine, $C(CH_2NH_2)_4$, obtained from E. I. du Pont de Nemours & Co. as a light-yellow oil boiling 96°–98°/4 mm., was condensed in absolute alcohol with four molecules of salicylaldehyde. Condensation occurred readily, but the orange-colored, oily product could not be made to crystallize. A cobalt derivative was prepared from a solution of the oil in alcohol. An insoluble, dirty-yellow cobalt derivative was obtained. This material was filtered off, dried at 100°, and alternately tested for oxygen-carrying capacity and treated at successively higher temperatures in a vacuum with the object of possibly rendering it active. All tests were negative and it was concluded that the material merited no further consideration.

DIETHYLENETRIAMINE AND SALICYLALDEHYDE

Diethylenetriamine, obtained from the Carbide and Carbon Chemicals Corporation as a light-yellow liquid, was distilled and a fraction boiling 203°–205°/740 mm. taken for use. A sample of this amine was titrated with standard hydrochloric acid using methyl red as indicater; assuming the amine to be diacidic, the analysis gave a purity of 105 per cent, indicating probably that a portion of the imino group also reacted with the acid.

Two equivalents of salicylaldehyde and one of diethylenetriamine were condensed in alcohol. No precipitate formed, and on evaporation a yellow viscous oil was obtained which could not be induced to crystallize. A solution of 15.5 g. of this oil in hot alcohol was added to an aqueous

solution of 12.5 g. of cobalt acetate. No precipitate formed, and on evaporation a tar was obtained which was dried and ground, yielding a brown powder. This material gained about 1 per cent in weight on exposure to air probably owing to the absorption of moisture. It did not absorb oxygen.

Other preparations of this cobalt compound, made by mixing cobalt acetate, salicylaldehyde and diethylenetriamine in water in the correct proportions gave the same inactive product.

DIETHYLENETRIAMINE AND 2-HYDROXY-3-NITROBENZALDEHYDE

A solution of 8.4 g. of 2-hydroxy-3-nitrobenzaldehyde, m.p.: 107°–108°, in 200 ml. of 50 per cent alcohol was prepared. When solution was complete, a quantity of 2.5 g. of diethylenetriamine in 50 ml. of water was added. Upon cooling in ice, orange crystals separated which were filtered off, washed with water, and dried. Yield: 8.7 g.; m.p.: 80°–81°.

To 4.01 g. of di-(2-hydroxy-3-nitrobenzal)-diethylenetriimine dissolved in a solution of 0.8 g. of sodium hydroxide in 100 ml. of water was added 4.5 g. of cobalt chloride dissolved in 50 ml. of water. A fine precipitate formed which was filtered off and dried in a vacuum at 100°. This material was dark orange in color. Yield: 2 g. When heated at temperatures ranging from 100° to 170° and followed by treatment with oxygen at 200 pounds pressure, an increase in weight of about 0.2 per cent was observed. No characteristic change in color occurred, the increase in weight being undoubtedly due to the absorption of moisture.

DIETHYLENETRIAMINE AND 2-HYDROXY-5-NITROBENZALDEHYDE

To 16.8 g. of 2-hydroxy-5-nitrobenzaldehyde, m.p.: 120°–121°, dissolved in 200 ml. of alcohol and 100 ml. of water was added 5.2 g. of diethylenetriamine in 100 ml. of water. Upon cooling, fine yellow crystals separated which were filtered off and washed with water. These crystals were then dissolved in 200 ml. of water containing 4 g. of sodium hydroxide. To the hot solution was added a solution of 11.89 g. of cobalt chloride in 50 ml. of water. A fine, light brown precipitate formed instantly which was filtered off and dried at 100° in a vacuum. Yield: 9 g. When heated and subjected to oxygen under pressure there was no apparent change in color or significant gain in weight.

TRIETHYLENETETRAMINE AND SALICYLALDEHYDE

Triethylenetetramine, obtained from the Carbide and Carbon Chemicals Corporation as a yellow liquid, was used without further purification. The condensation of salicylaldehyde with triethylenetetramine was effected in a hot alcohol solution. The Schiff's base could not be isolated as a crystalline product but only as a viscous, yellow oil. An alcohol solution of this Schiff's base was added with vigorous agitation to an aqueous solution of one equivalent of cobalt acetate. On boiling away a portion of the solvent, the product was recovered as a black tar which was removed, dried, and ground to a powder. The product was inactive toward oxygen.

TRIETHYLENETETRAMINE AND 2-HYDROXY-3-NITROBENZALDEHYDE

To 8.4 g. of 2-hydroxy-3-nitrobenzaldehyde dissolved in a solution of 100 ml. of alcohol and 50 ml. of water was added 3.6 g. of triethylenetetramine in 50 ml. of water. The solution became dark red, but even when cooled in ice no crystalline condensation product precipitated. After prolonged cooling a heavy dark red oil separated. The oil was separated, washed with water, and dried.

This Schiff's base in the form of a heavy oil was dissolved by adding the calculated equivalent of sodium hydroxide, assuming complete condensation to have taken place. To the hot solution was added the equivalent amount of cobalt chloride. A dark, turbid solution was obtained. After standing over night, a dark brown precipitate formed. This was filtered off and, although resembling other oxygen carriers in its purple color, it did not absorb oxygen.

TRIETHYLENETETRAMINE AND 2-HYDROXY-5-NITROBENZALDEHYDE

To 16.7 g. of 2-hydroxy-5-nitrobenzaldehyde dissolved in 100 ml. of alcohol and 50 ml. of water was added 7.3 g. of triethylenetetramine in 50 ml. of water. Granular yellow crystals separated which were filtered off and dried. Yield: 9 g.; m.p.: above 220°. To 4.44 g. of di-(2-hydroxy-5-nitrobenzal)-triethylenetetrimine dissolved in 150 ml. of water containing 0.8 g. of sodium hydroxide was added a solution of 4.53 g. of cobalt chloride in 50 ml. of water. A yellow-brown, rather granular precipitate formed instantly which was filtered off, and dried. It was heated at 150° in a vacuum and then subjected to oxygen at 200 pounds pressure. It did not gain in weight.

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STUDIES ON OXYGEN-CARRYING COBALT COMPOUNDS

X. Cobalt Derivatives of the Schiff's Bases of Salicylaldehyde with Alkylamines

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As shown in Paper IX of this series the cobalt derivatives of the Schiff's bases of salicylaldehyde with diamines other than ethylenediamine do not possess the property of absorbing oxygen. It was of interest to determine if the corresponding compounds using two equivalents of an alkylmonamine in place of ethylenediamine would be active toward oxygen.

In the case of the parent oxygen-carrying compound, bi-(disalicylalethylenediimine)-μ-aquo-dicobalt, it was only necessary to bring together, simultaneously, a cobalt salt, salicylaldehyde, and ethylenediamine, the formation of the Schiff's base and its cobalt derivative taking place together. This procedure failed with the alkylamines, the product being only cobalt disalicylaldehyde as indicated by the cobalt analysis and the absence of any amine in the product. By first preparing and isolating the Schiff's bases and then reacting an alcohol solution of the base with the cobalt salt it became possible to obtain the cobalt derivative. Even in this manner, however, the products were not always very pure. Evidently the Schiff's base underwent hydrolysis and the product in several cases contained some cobalt disalicylaldehyde. The latter, however, was usually sufficiently different in crystalline character and was precipitated after the other material had already formed so that a mechanical separation of the two was possible.

None of the disalicylalkylimine cobalt compounds prepared were active toward oxygen.

EXPERIMENTAL WORK

THE SCHIFF'S BASES OF ALKYLAMINES AND SALICYLALDEHYDE

The Schiff's bases of the lower alkylamines with salicylaldehyde are high-boiling, yellow oils insoluble in water. They were made by reacting an excess of salicylaldehyde with excess amine. The amine was added to the aldehyde slowly and with vigorous stirring until an excess of amine was present as indicated by the white fume produced when hydrochloric acid was brought near the solution. A yellow oil was obtained, and considerable heat was evolved. After cooling, the oil was washed several times with water to remove the excess of amine. The washings were extracted with benzene, and the benzene extract was added to the main

portion of the oily product. This mixture was then distilled at atmospheric pressure, water and benzene distilling first followed by pure benzene. The Schiff's base was then finally distilled under vacuum.

TABLE 1

PROPERTIES OF SALICYLALKYLIMINES

Alkylamine	Boiling Range of Schiff's Base	Color	Yield (percentage)
Methylamine	115°–117°/12 mm.	yellow	91
Ethylamine	129°–133.5°/20 mm.	deep yellow	85
n-Propyl	139.5°–142°/17 mm.	yellow	89
iso-Propyl	130°–132°/17 mm.	yellow	81
n-Butyl	150°–160°/20 mm.	reddish-yellow	91
n-Amyl	155°–164°/15 mm.	reddish-yellow	81

DISALICYLALMETHYLIMINE COBALT

To 0.1 mole of the Schiff's base, disalicylalmethylimine, dissolved in 50 ml. of absolute alcohol in a 3-necked, round bottom flask equipped with a mechanical stirrer and nitrogen inlet and outlet tubes, was added with constant stirring in a nitrogen atmosphere, a pulverized mixture of 0.05 mole of cobalt acetate and 0.1 mole of sodium bicarbonate. The stirring was continued until the evolution of carbon dioxide ceased. The flask was then heated and kept just below boiling for 15 minutes. Distilled water was then added to the dark solution until a tarry mass formed together with some dull, yellowish-green, solid precipitate. The latter was separated from the tar and solution, washed with distilled water, and dried in a vacuum at 70°. The cobalt in this material was determined by digesting a sample with nitric acid and sulfuric acid and followed by ignition and weighing as cobalt sulfate. Found: 18.5 per cent Co; theoretical for $C_{16}H_{16}O_2N_2Co$: 18.0 per cent Co.

DISALICYLALETHYLIMINE COBALT

This preparation was carried out using the same procedure employed above for the methyl compound. Two solid materials were obtained, brick-red lumps and fine, granular, yellowish-orange material. Both were dried in a vacuum at 70°. Found for the brick-red material: 15.0 per cent Co; found for the yellowish-orange material: 24.7 per cent Co. Theoretical for $C_{18}H_{20}O_2N_2Co$: 16.6 per cent Co. It is probable that the Schiff's base hydrolyzed partially and that the second material was largely cobalt disalicylaldehyde, the theoretical cobalt content of which is 19.6 per cent, contaminated by other cobalt-amine compounds.

DISALICYLAL-*n*-PROPYLIMINE COBALT

The same procedure was used. The orange-yellow product was dried in a vacuum at 80°. Found: 16.4 per cent Co; theoretical for $C_{20}H_{24}O_2N_2Co$: 15.4 per cent Co.

DISALICYLAL-*iso*-PROPYLIMINE COBALT

The same procedure was used. Red crystals separated from the solution during the reaction and it was not necessary to add water. Found: 15.88 per cent Co; theoretical for $C_{20}H_{24}O_2N_2Co$: 15.4 per cent Co.

DISALICYLAL-*n*-BUTYLIMINE COBALT

The same procedure was used. When water was added the solution gradually changed from dark brown to a milky yellow. A deep-red precipitate separated in the form of lumps followed by some finely divided orange-yellow material. These were separated from each other mechanically. Found for the red material: 13.17 per cent Co, for the orange-yellow: 17.0 per cent Co; theoretical for $C_{22}H_{26}O_2N_2Co$: 14.3 per cent Co. It is likely again that cobalt disalicylaldehyde was produced.

DISALICYLAL-*n*-AMYLIMINE COBALT

The same procedure was again used. On the addition of water two substances were thrown out of solution. The first was a semi-crystalline red mass and the other an orange-yellow powder. The former was probably the cobalt salt of the Schiff's base, but on drying in a vacuum it turned to a tar. The latter was probably cobalt disalicylaldehyde; found for it: 24.15 per cent Co.

DISALICYLALANIL COBALT

The same procedure was used again employing the Schiff's base of salicylaldehyde with aniline. During the course of the reaction a red, crystalline product separated. These crystals were filtered, washed with water, and dried in a vacuum. Found: 14.55 per cent Co; theoretical for $C_{26}H_{10}O_2N_2Co$: 13.1 per cent Co.

* * *

Further discussions of the "Studies on Oxygen-Carrying Cobalt Compounds" will follow in subsequent issues of the Journal of Science.

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